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BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. C. BAWDEN
J. W. EVANS
D. J. FINNEY
H. MARTIN

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STUDIES ON THE RELATIONSHIPS BETWEEN EARTHWORMS AND SOIL FERTILITY

IV. ON THE LIFE CYCLES OF SOME BRITISH LUMBRICIDAE

BY A. C. EVANS* AND W. J. McL. GUILD†
Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts

(With 6 Text-figures)

The rate of cocoon production in British Lumbricidae differs considerably from species to species and is greatly affected by soil temperature, moisture and the food supply of the adult worms. Only two out of fourteen species studied frequently produced more than one worm per cocoon, and twins were only recorded once in many hundreds of observations on four species of the genus *Lumbricus*.

The incubation period of the cocoons and the growth period to sexual maturity varies from species to species and also depends on the time of year at which the cocoons are deposited and the young worms emerge.

BREEDING TECHNIQUE

The technique finally adopted for maintenance of earthworm cultures was as follows. Five individuals were kept in a wide-mouthed screw-topped glass jar, the lid of which had the centre cut out and a piece of brass gauze substituted to prevent escape and permit ventilation. The jars were two-thirds filled with moist soil which had previously been thoroughly dried to kill any worms present, and a layer of moist bullock droppings, previously dried, put on top for food. Large species such as *Allolobophora longa* Ude and *A. nocturna* Evans were kept in 3-pint jars and the smaller species in 500 g. chemical jars. This technique proved very successful for all species except *Lumbricus terrestris* L., the largest British earthworm, which remained in good condition in 3-pint jars but produced few cocoons. Successful cocoon production was finally achieved by keeping three individuals in 5-pint jars.

The cultures were kept in an underground chamber (an air-raid shelter) with seasonal variations in temperature resembling those of the soil at a depth of 4 in. but rather higher in winter and lower in summer.

Rates of reproduction were obtained by transferring the worms to fresh soil and bullock droppings every fortnight, the cocoons being recovered from the soil by washing it through a sieve of suitable mesh with a fairly strong spray of water. The cocoons of most species are readily discernible in the debris left on the sieve, their 'lemon' shape and yellow or greenish yellow colour being most distinctive. Some difficulty was encountered at first in recovering quantitatively the cocoons of *Allolobophora caliginosa* Sav. and *Lumbricus rubellus* Sav. since the soil adhered

* Now at United Africa Co. (M.A.) Ltd., P.O., Kongwa, Tanganyika.
† Now at Department of Agricultural Zoology, 10 George Square, Edinburgh.

tenaciously to them, and prolonged washing was necessary to ensure quantitative recovery. The cocoons were counted and weighed, then kept in beakers containing clean sand and water. In this way the development of the worms in the cocoons could be kept under observation and the date of emergence determined accurately. On emergence the worms were transferred, singly, to glass tubes, 3 × 1 in., capped by a short piece of rubber tubing of $\frac{7}{8}$ in. diam. into which a piece of brass gauze was sealed with rubber solution. Sixty individuals of most species were set up in this way and examined at intervals to determine the time when they became sexually mature. Individuals of the small species such as *L. castaneus* Sav. and *Dendrobaena subrubicunda* Sav. were reared to maturity in these tubes, but those of the larger species were transferred at an appropriate size to larger jars varying in size from $\frac{1}{2}$ lb. honey jars to 5-pint jars according to the ultimate size of the species.

Cocoons

The worms which were used for cocoon production were obtained from field populations and so consisted of worms of varying and unknown ages.

Seasonal changes in cocoon production

Data on the seasonal rates of reproduction were obtained during 1945 and 1946 for many of the common species, and curves illustrating the fortnightly production of cocoons by ten species under approximately natural conditions of soil temperature but at optimum soil moisture are given in Fig. 1. The temperature of the two seasons (March-November) differed slightly, the mean for 1945, 11·6° C., being 0·9° higher than that of 1946. During March to July 1945 the temperature was about 1° C. higher than during the same period in 1946. This period, as will be shown below, is more important for cocoon production than August to October. In spite of these differences the total cocoon production was the same in both years for *Allolobophora caliginosa* and *A. chlorotica* Sav. but that of *Lumbricus rubellus* was reduced by 25% in 1946.

Fig. 1 shows the changes in rates of reproduction for ten species of earthworms, some for 1945, some for 1946 and in *L. rubellus* for both years. The curves clearly show that there are considerable seasonal and absolute differences between the various species. Two species, *Allolobophora longa* and *A. nocturna*, reproduced only from mid-March to June-July and again from October to November. The interruption in reproduction during the summer months was brought about by an obligatory diapause. This diapause is a characteristic feature in the life cycle of the adults of these species and occurs in summer, even though conditions of temperature, moisture and food supply are suitable for continued reproduction. The worms coil up in a spherical cell in the soil and the secondary sexual characters regress to such an extent that they can only just be discerned (Avel, 1929). Six species, *Lumbricus rubellus*, *Allolobophora caliginosa*, *A. chlorotica*, *Eisenia foetida* Sav., *E. rosea* Sav. and *Octolasium cyaneum* Sav., did not show any interruption in reproduction once

the threshold temperature had been passed, and their rates of reproduction followed the temperature curve fairly closely. The reproduction rates of three species, *Lumbricus castaneus*, *Dendrobaena subrubicunda* Sav. and *D. mammalis* Sav., resembled those of the above six species during the first part of the season but differed in that they ceased reproduction earlier due to death of the worms. Evidently the longevity

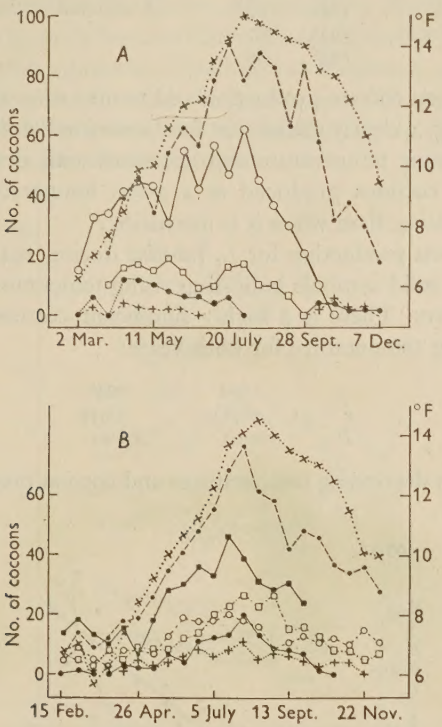

Fig. 1. Fortnightly production of cocoons by ten individuals of ten species of earthworms. ×···×, temperature; ●−−●, *L. rubellus*; A: ○—○, *L. castaneus*; □—□, *D. mammalis*; ●···●, *A. longa*; +−−+, *A. nocturna*. B: ■—■, *D. subrubicunda*; □−·−□, *A. caliginosa*; ○−−○, *A. chlorotica*; ●—●, *E. foetida*; +···+, *E. rosea*.

of these three species is less than that of the other species studied. Considerable differences in the number of cocoons produced per individual per annum by the different species were found (see Table 1). These results must only be taken as indicating that considerable differences do exist in the rate of cocoon production of the different species. Data obtained in the course of other work suggests that *Lumbricus rubellus*, *L. castaneus*, and *Dendrobaena subrubicunda* are characterized by a high reproductive rate and *Lumbricus terrestris*, *Allolobophora longa* and *A. nocturna* by a low reproductive rate, but much further work remains to be done on individuals of known age before accurate comparisons can be made.

TABLE 1. *Number of cocoons produced per annum by one individual*

L. rubellus	1945	106	*D. mammalis*	1945	17
	1946	79	*O. cyaneum*	1946	13
L. castaneus	1945	65	*E. foetida*	1946	11
D. subrubicunda	1946	42	*E. rosea*	1946	8
A. caliginosa	1945	27	*A. longa*	1945	8
	1946	27	*A. nocturna*	1945	3
A. chlorotica	1945	27			
	1946	25			

The relation between cocoon production and temperature over the whole year is not a simple one. Fig. 1 clearly shows that for *Lumbricus rubellus* cocoon production increases with increasing temperature and decreases with decreasing temperature, but the number of cocoons produced at a given temperature is less when the temperature is decreasing than when it is increasing.

In Fig. 2 the cocoon production for *L. rubellus* during 1945 and 1946 is plotted against temperature, solid symbols indicating rising temperature and outlined ones decreasing temperature. There is a highly significant correlation between cocoon production and rising temperature for both years:

	1945	1946
r	0·963	0·972
P	<0·01	<0·01

The relation between decreasing temperatures and cocoon production appears to be curvilinear.

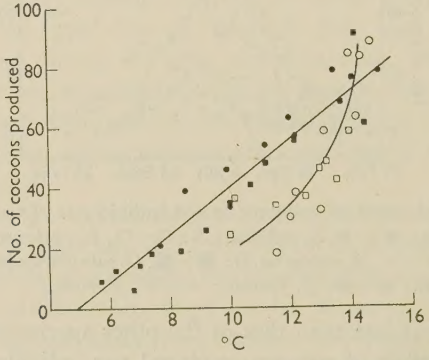

Fig. 2. Relationship between cocoon production by *L. rubellus* and increasing and decreasing temperature.

1945	1946	
●	■	increasing temperature
○	□	decreasing temperature

Similar results were obtained for *Allolobophora caliginosa*, *A. chlorotica*, *Eisenia rosea* and *Octolasium cyaneum*. These species together with *Lumbricus rubellus* survived without any appreciable mortality during the year. It may be that after

a period of rapid reproduction a kind of 'fatigue' of the reproductive system sets in and a period of comparatively slow reproduction follows during which recovery occurs. This type of relationship between temperature and reproduction may occur in certain habitats which are moist throughout the year. In others, in which the soil may become too dry for activity during the summer, it may not occur, as reproduction is interrupted in the genera *Allolobophora*, *Eisenia*, and *Octolasium* by a facultative diapause in such dry conditions (Evans & Guild, 1947). In the case of three species, *Lumbricus castaneus*, *Dendrobaena mammalis* and *D. subrubicunda*, which died during the year, the rate of reproduction fell appreciably some weeks before death occurred. The worms also diminished appreciably in size as did the cocoons several weeks before death occurred.

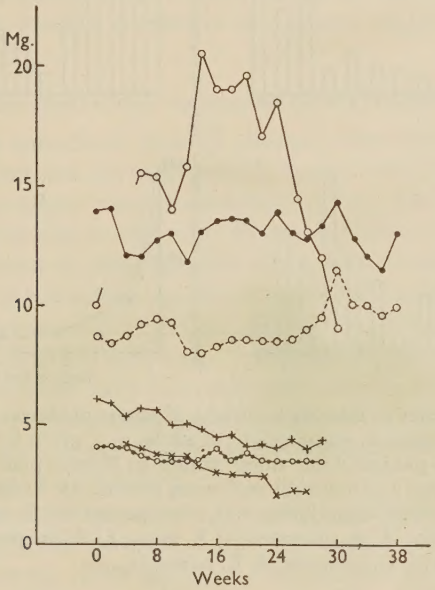

Fig. 3. Seasonal changes in weight of cocoons of six species of earthworms. ○—○, *E. foetida*; ●—●, *L. rubellus*; ○--○, *A. chlorotica*; +—+, *D. subrubicunda*; ○-·-○, *L. castaneus*; ×—×, *D. mammalis*.

Seasonal changes in weight

During the first year's work most of the cocoons produced in seasonal study of reproduction were used to obtain data on the weights of the cocoons of the different species. The results showed that the cocoons of certain species remained fairly constant, but those of other species showed changes in weight during the year. The cocoons produced during the second year's work were weighed in groups and the mean weights per cocoon showed similar trends to those found during the first year (Fig. 3).

The cocoons of eight species remained fairly constant in weight throughout the year, i.e. *Allolobophora caliginosa*, *A. chlorotica*, *A. longa*, *A. nocturna*, *Eisenia rosea*, *Lumbricus castaneus*, *L. rubellus* and *Octolasium cyaneum*. Those of *Eisenia foetida* increased in weight until the beginning of June when they reached their maximum weight of about 20 mg., remained at this level until mid-July and then decreased in weight rapidly until cocoon production ceased in early October. The cocoons of two species, *Dendrobaena subrubicunda* and *D. mammalis*, showed a progressive decline in weight from the beginning of the year until the cultures died out.

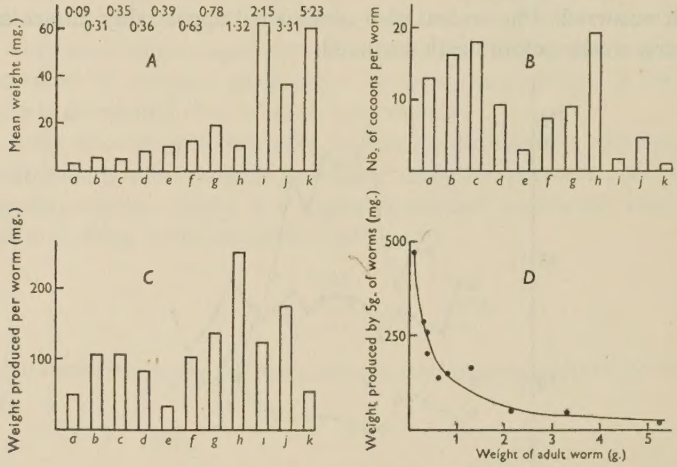
Mean weight (mg.)
0·09 0·35 0·39 0·78 2·15 5·23
0·31 0·36 0·63 1·32 3·31
A
No. of cocoons per worm
B
Weight produced per worm (mg.)
C
Weight produced by 5g. of worms (mg.)
D
Weight of adult worm (g.)
a b c d e f g h i j k

Fig. 4. Cocoon production in relation to weight of adults of eleven species of earthworms. A: mean weight of cocoon, mean weight of adults in g. given for each species. B: mean number of cocoons produced per worm during an 8-week period. C: mean weight of cocoons produced per worm during an 8-week period. D: weight of cocoons produced by 5 g. of the different species during an 8-week period. *a*, *D. mammalis*; *b*, *D. subrubicunda*; *c*, *L. castaneus*; *d*, *A. chlorotica*; *e*, *E. rosea*; *f*, *A. caliginosa*; *g*, *E. foetida*; *h*, *L. rubellus*; *i*, *A. longa*; *j*, *O. cyaneum*; *k*, *L. terrestris*.

Relations between size of worm and weight of cocoon

It might be expected that the weight of the cocoon would bear a close relationship to the size of the species producing it. Early on in the work it became evident that this was not so, certain large species producing much smaller cocoons than expected. The worms in the cultures were weighed and the numbers and weights of cocoons produced during the subsequent 8 weeks were related to those weights. Fig. 4 shows the results obtained over this period.

Three of the species, *Lumbricus terrestris*, *L. rubellus* and *Octolasium cyaneum* produce smaller cocoons relative to their size than do the remaining species (Fig. 4*a*). The number of cocoons produced during the 8-week period was not related to size, but the three smallest species produced more cocoons than the larger species with

the exception of *Lumbricus rubellus* which produced the highest number (Fig. 4*b*). Similarly, the total weight of cocoons produced was not related to the species size (Fig. 4*c*). *Dendrobaena mammalis*, the smallest species, weighing only 90 mg., produced the same weight of cocoons as *Lumbricus terrestris*, the largest species, weighing over 5 g. If the weight of cocoons produced by a given weight of worms of each species is calculated for the 8-week period (Fig. 4*d*), it is seen that the smaller species produce a much greater weight of cocoons than the larger species, the points relating weight of cocoons falling on a hollow curve. Two species do not fit closely to this curve, *Eisenia rosea* falling below it and *Lumbricus rubellus* above it.

It is possible that a further extension of this type of work may lead to results of value in assessing the relative activities of field populations with differing proportions of species in breaking down organic matter in the soil, a lower weight of smaller species being possibly as effective as a greater weight of larger species.

The effects of soil moisture on cocoon production

The results so far considered were all obtained from worms kept in soil at an optimum water content and the only variable factors were temperature and age of worms. Since soil moisture varies considerably during the year and affects worm activity and cast production considerably (Evans & Guild, 1947), it was decided to carry out an experiment to determine the effects of soil moisture on cocoon production. Evans (1944) has shown that soil moisture expressed as a percentage of the dry soil is not a suitable measure for expressing the relationship between loss of weight of wireworms and soil moisture. A more suitable measure is Schofield's *pF*. Experiments were therefore carried out using two different soils, a clay soil obtained from Bones Close field at Rothamsted and a gravelly soil obtained from the Westfield allotments at Harpenden, to see whether *pF* is a suitable measure for expressing the soil moisture relations of earthworms.

After some preliminary observations, jars containing Bones Close soil at 11, 13·5, 21, 28, 35·5 and 42·5% soil moisture and Westfield soil at 16, 24·5, 33, 42 and 50% soil moisture were set up. Five replications of each soil moisture, each containing five individuals of *Allolobophora chlorotica* with plump clitella were set up. The worms were allowed to reproduce for a period of 43 days during which the temperature dropped slowly from 10 to 8° C. They were then removed and the cocoons washed out of the soil, counted and their fresh and dry weights determined. It was noticed one week after the experiment had started that the worms on Bones Close soil 11 and 13·5 and Westfield 16% soil moisture were either coiled up in a cell in the soil or partially so, while at the remaining soil moistures they were active and remained so throughout the experiment.

Table 2 gives the results obtained in the experiments on a percentage soil-moisture basis. Within each soil type, moisture clearly has a big effect on cocoon production. At the lowest soil moistures, those causing rolling up of the worms, no

cocoons were produced, but even at the four soil moistures which permit activity there are highly significant differences in the number and size of the cocoons produced. In the Bone Close soil, the maximum number of cocoons was found at 28% soil moisture, and increase and decrease of soil moisture from this figure caused significant diminutions in cocoon production. In the Westfield soil, the maximum number of cocoons were produced at 33 and 42% moisture with a significant decrease at 24·5 and 50%. There are clearly big differences between cocoon production at similar moisture contents on the two soils. At 21% moisture for Bones Close cocoon production was fourteen times higher than that at 25% for Westfield soil. The mean fresh weight and dry weight of the cocoons increases with increasing soil moisture. The percentage dry weight of the cocoons was fairly constant and showed no relationship to the variable moisture content of the two soils.

Table 2. *The effect of soil moisture on cocoon production*

Bones Close							
Moisture content of soil	11	13·5	21	28	35·5	42·5	S.E. of difference
Mean no. of cocoons produced by five worms	0	0	8·6	13·6	8·8	6·6	0·94
Mean fresh weight of cocoons (mg.)	—	—	10·0	10·8	13·7	16·4	0·56
Mean dry weight of cocoons (mg.)	—	—	2·56	2·69	3·27	3·80	0·27
% dry weight of cocoons	—	—	25·6	23·1	24·0	23·1	—
Westfield							
Moisture content of soil	—	16	24·5	33	42	50	S.E. of difference
Mean no. of cocoons produced by five worms	—	0	0·6	8·4	9·4	3·0	0·93
Mean fresh weight of cocoons (mg.)	—	—	9·4	10·9	11·5	15·2	1·61
Mean dry weight of cocoons (mg.)	—	—	—	2·61	2·57	3·89	0·32
% dry weight of cocoons	—	—	—	24·0	22·5	25·6	—

The *pF* values of the moisture contents were read from curves relating *pF* and soil moisture for the two soils. In Fig. 5 the results for cocoon production and fresh weight of cocoon are plotted against percentage soil-moisture content and against the appropriate *pF* values. When plotted against percentage soil moisture, the curves for the two soils are quite distinct. When plotted against *pF*, the points for cocoon production tend to fall on to one curve, and the points for fresh weight of cocoon definitely fall on to one curve. For earthworms as for wireworms, it is not the amount of water present in the soil that determines rate of reproduction or size of cocoons but the force with which the water is held by the soil.

Two further physical properties of the soils were examined in relation to cocoon production, namely, the plastic point and the sticky point. These points are marked in Fig. 5. It can be seen that cocoon production is low in the region of the plastic point and does not reach a maximum until the moisture content of the soil is greater than that at the sticky point. It will be necessary to extend these observations to

a greater range of soil types before it can be decided whether what can be loosely termed the 'palatability' of the soil has an effect on cocoon production or whether it is only the low humidity occurring in the pore-spaces that depresses the rate of cocoon production. At $pF3$ the relative humidity of the air in the soil is 99·3%.

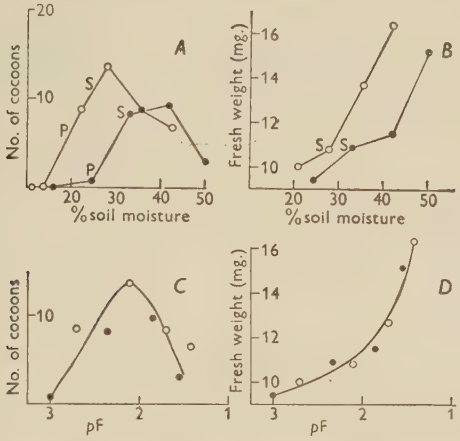

Fig. 5. Cocoon production in relation to soil moisture. ○ = Bones Close, ● = Westfield; P, plastic point; S, sticky point. *A*, numbers in relation to percentage soil moisture. *B*, mean fresh weight in relation to percentage soil moisture. *C*, numbers in relation to *pF*. *D*, mean fresh weight in relation to *pF*.

The effect of the nutrition of adults on cocoon production

The amount and type of organic matter available as food for earthworms varies enormously according to the habitat. Very little is known about the food requirements and preferences of the several species and nothing has been published on the effect of different types of organic matter on cocoon production.

An experiment was set up to determine the effect of several types of organic matter, at various stages of decomposition, on the cocoon production of two species of earthworms, *A. chlorotica* and *Lumbricus castaneus*. Nine types of organic matter were used: sewage sludge, well-rotted farmyard manure, peat from a peaty pasture, bullock droppings, horse droppings, sheep droppings, cut grass from pasture from which the animal droppings were obtained, oat straw, oat straw + 1·5 g. of sulphate of ammonia per 12 g. straw. Five replicates of each treatment were set up.

The worms were allowed to feed for 12 weeks before the cocoons were washed from the soil. Table 3 gives the results obtained.

The results for *L. castaneus* were analysed on a square root transformation because of the big differences obtaining between the means of the different treatments and statements made below on the significance of treatment differences are based on this analysis. The types of organic matter used fall into three groups, originally undecayed (two straw treatments and fodder), partially decayed (animal

droppings and peat), and well decayed (sewage sludge and farmyard manure). The cocoon production of both species falls into two groups, high and low. High production occurred on the partially decayed types of organic matter and low production on the originally undecayed and the well decayed types. The differences are clearly significant.

Table 3. *The cocoon production of two species of earthworms on various types of organic matter*

Organic matter	Mean number of cocoons produced by five earthworms	
	A. chlorotica	*L. castaneus*
Sewage sludge	0·8	8·4
Farmyard manure	0·2	8·6
Peat	10·0	28·2
Bullock droppings	12·4	73·2
Horse droppings	12·6	55·2
Sheep droppings	14·0	76·0
Fodder	0·8	9·4
Oat straw	1·4	12·0
Oat straw + sulphate of ammonia	0·4	19·2
S.E. of difference	±1·4	

Oat straw is poor in nitrogen compounds and was expected to produce a low cocoon production, and the addition of sulphate of ammonia had neither a beneficial nor an adverse effect. The low value resulting from the fodder was quite unexpected, particularly as the animal droppings derived from it produced high values. Farmyard manure also produced unexpectedly low values. The low values produced by this and sewage sludge may possible be due to the low amount of digestible carbohydrate present, since in another experiment it was found that the addition of ground straw to bullock droppings appreciably increased cocoon production. In this experiment the effect of the addition of 2 and 4 g. of ground straw to 4 g. of bullock droppings resulted in nearly 100% increase in cocoon production.

	Without straw	2 g. straw	4 g. straw	S.E. of difference
Cocoon production by three *A. chlorotica* in 4 months	19	35	36	±2·3

The number of worms emerging from individual cocoons

The number of ova in a cocoon varies in different species, from one or two up to twenty according to the species (Stephenson, 1930). It was considered that a study of the variation in the number of young worms emerging from the cocoons of the different species would be of interest and of value in interpreting population changes in the field.

A number of cocoons of the different species were set up individually in glass tubes at different times over a period of 2 years. Results were obtained on fifteen

species and are shown in Table 4. Considerable differences are seen to exist between the different species. One to four worms usually issue from one cocoon of *Eisenia foetida;* one or two from *Dendrobaena subrubicunda* and one from the remaining species. *Eisenia foetida* and *Dendrobaena subrubicunda* are the only species recorded as having produced more than two worms from one cocoon, the former up to as many as eight, the latter up to four. The genus *Lumbricus* differs markedly from the other five genera studied in that only one of its species has produced more than one worm from one cocoon. This appears to be characteristic of the genus since although emergences of many hundreds of cocoons of *L. castaneus* have been observed, the emergence of two worms from one cocoon has never been recorded and only one out of many hundreds for *L. rubellus*. Generally only one worm issued from the cocoon of the four species of *Allolobophora* studied, the percentage of cocoons giving rise to two worms being quite small. In *Eisenia* there is a great contrast between the two species studied, *E. foetida* normally producing more than one worm per cocoon and *E. rosea* only one. Whether the proportion of cocoons giving rise to two worms is greater in *Octolasium cyaneum* than in *O. lacteum* cannot yet be determined, as only one batch of cocoons obtained from a few individuals of the latter species has been examined.

TABLE 4. *Variations in the number of worms emerging from isolated cocoons expressed as a percentage of viable cocoons*

Species	No. of viable cocoons	Percentage cocoons producing 1–8 young worms							
		1	2	3	4	5	6	7	8
A. longa	89	97	—	—	—	—	—	—	—
A. nocturna	56	93	7	—	—	—	—	—	—
A. caliginosa	131	100*	—	—	—	—	—	—	—
A. chlorotica	142	100*	—	—	—	—	—	—	—
D. subrubicunda	340	62	33	4½	½	—	—	—	—
E. foetida	139	20	30	27	15	2	3	2	½
E. rosea	42	100*	—	—	—	—	—	—	—
E. tetraedra	198	95½	4½	—	—	—	—	—	—
L. castaneus	182	100	—	—	—	—	—	—	—
L. festivus	50	100	—	—	—	—	—	—	—
L. rubellus	105	100*	—	—	—	—	—	—	—
L. terretris	82	100	—	—	—	—	—	—	—
O. cyaneum	40	90	10	—	—	—	—	—	—
O. lacteum	50	100	—	—	—	—	—	—	—

* Indicates that two worms have been observed very occasionally to issue from one cocoon in batches of cocoons kept for obtaining information on incubation periods.

There appears to be a possibility that the proportion of cocoons giving rise to more than one worm is a racial characteristic. Kleinenberg (1879), studying *Allolobophora caliginosa f. trapezoides* Ant. Duges, regarded the emergence of two worms from one cocoon as being normal. In this case the two worms were true

twins, being derived from one egg. Weber (1917) examined 184 cocoons of this variety and found that:

1 contained 4 embryos;
101 contained 2 individuals;
57 contained 1 individual;
25 contained eggs in various cleavage stages.

Thirty-five of the 101 cocoons containing two individuals had various kinds of 'monsters', i.e. incompletely separated worms. Vejdovsky (1890) maintained that the development of two embryos from one egg is abnormal.

In this study, *f. typica* was used and it can be seen that the emergence of two worms from one cocoon is definitely unusual.

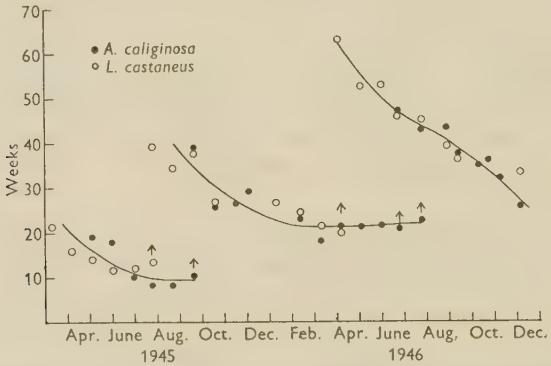

Fig. 6. Incubation periods of cocoons of *A. caliginosa* and *L. castaneus* deposited between March 1945 and December 1946.

Incubation periods

Very few data are available on the period of development in the cocoon. Wilson (1889) found that emergence of *Eisenia foetida* occurred in 2–3 weeks and Hoffmeister (1845) records 3 weeks for *Octolasium cyaneum*. Both investigators kept the cocoons at laboratory temperatures.

Numerous records of incubation periods for the common species have been obtained during the present investigations. Owing to the very great variations which occurred at certain periods and seasonal gaps in the data a detailed comparison of the species and of the effects of temperature cannot be made. Fig. 5 shows the relation between the times at which the cocoons of *Allolobophora caliginosa* and *Lumbricus castaneus* were deposited and the incubation periods. At certain times, March to June 1945, and October 1945 to March 1946, the incubation periods of the two species are very similar. At other times, August 1945 and May 1946, there are very great differences amounting to 24 and 32 weeks respectively. Certain batches of cocoons (marked with an arrow in Fig. 6) for both species

clearly had two distinct emergence groups, an interval of 10–12 weeks intervening between the emergence of the last individual of one group and the first of the other group. This interval coincided with the period of low temperature during winter. For *L. castaneus* this division of a batch of cocoons deposited during a fortnight into two emergence groups occurred earlier in the year than for *Allolobophora caliginosa*, and this suggests that the threshold of development temperature is higher for *Lumbricus castaneus*.

Comparative figures for the incubation periods of several species are given in Table 5.

TABLE 5. *Incubation periods for cocoons deposited between 13 April and 11 May* 1945

Species	Weeks	Species	Weeks
A. caliginosa	19	*E. rosea*	17½
A. chlorotica	12½	*L. castaneus*	14
A. longa	10	*L. rubellus*	16
D. mammalis	15	*O. cyaneum*	21
D. subrubicunda	8½	*O. lacteum*	21
E. foetida	11		

The majority of the worms emerged from the cocoons within a fortnight. The differences between the species is not very great and bears no relation to the size of the species, neither are there any striking generic differences.

GROWTH PERIODS FROM EMERGENCE TO SEXUAL MATURITY

The data on growth periods are also irregular, as cultures had to be set up when newly emerged worms were available. The results obtained are given in Table 6.

TABLE 6. *Growth periods in weeks of several species*

Emerged	*A. caliginosa*	*A. chlorotica*	*A. longa*	*D. subrubicunda*	*E. rosea*	*E. foetida*	*L. castaneus*	*L. rubellus*
Nov. 1944	—	42	—	—	—	74	—	—
Dec.	—	39	38	—	—	—	—	—
Jan. 1945	—	35	—	33	—	70	—	—
Feb.	—	30	—	29	—	74	—	—
Mar.	—	29	—	27	—	—	—	—
Apr.	58	—	71	27	—	—	—	—
May	53	—	70	22	62	—	—	—
June	56	—	64	—	61	—	—	—
July	54	—	58	—	53	47	18	37
Aug.	—	—	54	—	50	—	—	—
Sept.	—	—	51	—	—	—	—	—
Feb. 1946	—	—	40	42	—	—	—	—
Mar.	—	—	—	38	—	—	25	—
Apr.	—	—	—	33	—	—	25	—
May	—	—	—	32	—	—	25	—

Several species, *Allolobophora caliginosa*, *A. longa*, *Eisenia rosea* and *E. foetida* took approximately one year to reach sexual maturity, *Allolobophora chlorotica* and *Dendrobaena subrubicunda* from 7 to 10 months and *Lumbricus castaneus* about

6 months. *L. rubellus*, a larger species than *L. castaneus*, took about twice as long to reach sexual maturity.

Periods of diapause during growth were not observed in *Dendrobaena subrubicunda*, *Eisenia foetida*, *Lumbricus castaneus* or *L. rubellus*. The three species of *Allolobophora* and *Eisenia rosea* entered into a diapause during the colder winter months.

We are indebted to Mr M. V. Brian, for carrying out the experiment on the effects of soil moisture on cocoon production and to the Agricultural Research Council for a grant to one of us (W. J. McL. G.).

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STUDIES ON THE RELATIONSHIPS BETWEEN EARTHWORMS AND SOIL FERTILITY

V. FIELD POPULATIONS

By A. C. EVANS* and W. J. McL. GUILD†
Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts

(With 4 Text-figures)

The ploughing of a permanent pasture in spring was not followed by a reduction of the earthworm population during the first 6 months but further arable cultivation did greatly reduce the population. Leys, 4–7 years old, carry a total population similar to that of permanent pastures, but with differing proportions of certain species. Some trends in population changes during a period of nearly 1 year are described for five common species. The population of a permanent pasture did not show appreciable change for a period of nearly 3 years but that of a ley, in its first year following arable, showed a significant increase.

Introduction

Evans & Guild (1947) showed that the population and proportions of the different species of earthworms in a field is greatly influenced by the previous agricultural history of the field. The conclusions were based only on the adult earthworms, and it was clear that the proportions of adults to immature worms differed from species to species. A technique has been developed which permits the identification of 95–100% of the earthworms in a sample, except for distinguishing immature specimens of *Allolobophora longa* Ude from those of *A. nocturna* Evans, the adults of which are closely related. The data recorded as *A. nocturna* refers to the total for the two species, *A. longa* adults being present only in very small numbers.

The size of plot and technique for bringing the worms to the surface were the same as in the previous work. Nine of the twelve fields sampled have been described in the second paper of this series (Evans, 1948). Descriptions of the other three are given below:

Appletree: fourth year grass following 2 years arable, previously permanent grass.

Longcroft: sixth year grass, reclaimed from woodland felled before 1939.

Delharding: in stubble after winter oats, fifth cereal crop, previously permanent grass.

The sampling was carried out between 21 and 25 October 1946 when soil conditions were very favourable.

* Now at United Africa Co. (M.A.), Ltd., P.O., Kongwa, Tanganyika.
† Now at Department of Agricultural Zoology, 10 George Square, Edinburgh.

Comparative populations on fields of varying agricultural history

The twelve fields can be conveniently divided into five main types: permanent pasture; previous permanent pasture, reseeded after 2 years arable; leys after woodland or arable; first year in arable; and fifth year in arable. Table 1 shows the results expressed as fiducial limits of the means, that is to say it is a twenty to one chance that the true mean will lie within these limits, and if the limits for two populations do not overlap then there is a significant difference between their means.

Table 1. *The effects of previous agricultural history on earthworm populations of several fields; weight in lb./acre, populations in* 1000's*/acre*

	Weight	Total Population	*A. nocturna*	*A. caliginosa*	*E. rosea*	*L. terrestris*	*A. chlorotica*
Permanent pastures	646–456	459–285	228–141	141–60	33–14	58–35	17– 4
Permanent pastures ploughed, arable, reseeded.	423–315	266–192	78– 45	85–60	32–19	54–30	44–14
Leys	643–443	323–219	61– 34	92–70	28–14	97–68	—
First year arable	746–362	330–230	198–109	67–38	18– 5	53–25	20– 3
Fifth year arable	125– 61	129– 69	31– 13	46– 2	29– 7	4–?	32–10

There are clearly significant differences between the populations of the various agricultural treatments, and also between those of the different species on any one treatment. The spring ploughing of permanent pasture does not bring about a significant reduction in either weight, total population or any one species in the first season. After 5 years arable there is a great reduction in weight and population of all species except *A. chlorotica* Sav. and *Eisenia rosea* Sav., a reduction of approximately 80% in weight and 70% in numbers. This reduction is brought about by the decrease in available food and, perhaps also for the two large species, *Allolobophora nocturna* and *Lumbricus terrestris* L., by the changed soil conditions. When such fields are grassed down after only two arable crops have been taken, the population, after 2 years under grass, is found to have increased again. The weight and total population are just significantly lower than those of permanent pastures and the difference is accounted for by the slower recovery of *Allolobophora nocturna*, the other species showing no significant differences. The leys carry high populations but are characterized by a low proportion of *A. nocturna* and by a high proportion of *Lumbricus terrestris*. Data on *Allolobophora chlorotica* in leys is not given as two of the three leys are situated on the western edge of the farm and this species is rarely found there. The reason for this is not known.

The highest population is present on a 300-year-old pasture, 770–523,000/acre, and the weight of earthworms present lies between 9 and 13 cwt./acre. This field also carries the highest population of *A. nocturna*. The lowest population estimated is present on the 5-year arable field, 129–69,000/acre, and the weight lies between $\frac{3}{4}$ and $1\frac{1}{2}$ cwt./acre.

TABLE 2. *Ratios of immature worms to adults*

Field	*A. nocturna*	*A. caliginosa*	*A. chlorotica*	*E. rosea*	*L. terrestris*
Parklands	4·5	3·0	0·5	2·2	10·3
Great Field I	1·9	1·4	0·5	1·9	12·3
Great Field III	2·5	1·5	0·3	1·8	11·1
Pastures	3·1	0·7	0·2	1·2	7·3
Longcroft	2·3	1·6	0·6	3·4	6·9
Claycroft	1·5	1·1	—	1·8	7·5
Appletree	5·3	1·0	0·5	2·1	5·0
Great Field II	4·5	1·2	0·3	2·5	19·3
Parklands Wedge	3·5	1·5	—	5·0	12·9
New Zealand	2·0	1·7	0·3	1·7	—
Stackyard	12·8	2·3	0·6	3·0	5·7
Delharding	3·3	2·5	1·6	1·9	1·5

Table 2 shows that the proportions of adult to immature worms varies considerably according to the species but not, with a few exceptions, from field to field at the time of year when the sampling was done. All fields, except the 5-year arable Delharding, show more adult *A. chlorotica* than immature, about 3:1. All the other species on all fields, except *A. caliginosa* on Pastures, show more immatures than adults. *A. caliginosa* and *Eisenia rosea* have about two immatures per adult and *Allolobophora nocturna* about three per adult except on Stackyard where it is thirteen per adult. This figure is based on thirty adults and 385 immatures from seventeen samples and so is reliable. It is possible that the spring ploughing and subsequent cultivations directly destroyed a much higher proportion of the adults than of the immatures. Only two adults per sample were taken compared with six to nine on the three permanent pastures, but twenty-three immatures per sample were present compared with thirteen to forty-one for the permanent pastures. Also samples taken in spring 1945 showed eight adults per sample, but the number of immatures is not known since they could not be accurately identified at that time. The highest proportion of immatures to adults is shown by *Lumbricus terrestris*, 11–12:1, on some fields, i.e. permanent pastures and 2–4 years grass after 2 years arable, 7:1 on leys and 1·5:1 on 5-year arable. Since it is possible that spring ploughing on Stackyard killed off a high proportion of the adults of *Allolobophora nocturna* it is surprising that the proportion of immature to adult *Lumbricus terrestris*, a larger species than *Allolobophora nocturna*, is as low as 6:1. In spring 1945 the ratio of immature to adult *Lumbricus terrestris* was 5:1. It is possible that *L. terrestris* was able to retreat more deeply into the soil than *Allolobophora nocturna* and so escape severe mechanical damage. It has been noticed during sampling that *A. nocturna* generally appears on the surface before *Lumbricus terrestris*, suggesting that it is working closer to the surface.

Changes in population during the period August 1946 *to June* 1947

A series of samplings was carried out on Parklands to determine comparative changes in populations of five common species of earthworms. This field was chosen

because of its high population and because adults of *Allolobophora longa* were only to be found very rarely, and so over 99% of the immature worms identified as *A. nocturna* really belonged to this species. In order to minimize the effects of soil moisture and temperature on the results, sampling was done only on days favourable for activity as far as could be arranged.

The five species can be arranged in three groups each differing somewhat in the trend of population during the 10-month active season (see Fig. 1). *Lumbricus terrestris* showed no changes in population from 15 August to 2 December; there was a sharp drop in the numbers per sample during the winter but the original

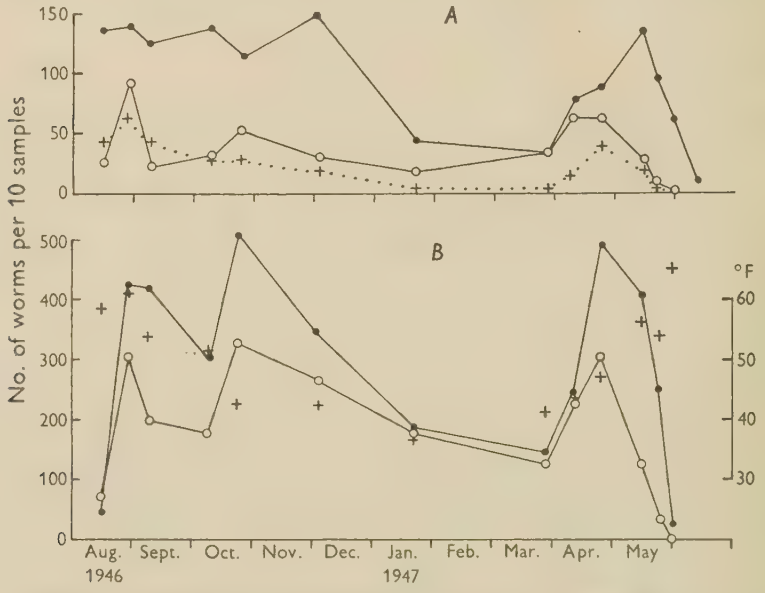

Fig. 1. Seasonal trends in total populations. A: ●—●, *L. terrestris*; ○—○, *E. rosea*; +...+, *A. chlorotica*. B: ●—●, *A. nocturna*; ○—○, *A. caliginosa*; +, temperature.

value was regained by 16 May. This suggests that the winter decrease was largely due to lower temperatures. The decrease in numbers after 16 May was due to decrease in soil moisture. *Allolobophora chlorotica* and *Eisenia rosea* reached maximum numbers on 28 September and then declined. This decline is probably a real decrease in population; both species work right up in the soil surface when conditions are favourable and so are particularly vulnerable to surface predators. Both species show an increase in numbers in spring, probably due to emergence from overwintered cocoons as the increase is chiefly accounted for by immature individuals. Decrease in soil moisture accounted for the decline in numbers after 25 April. *Allolobophora nocturna* and *A. caliginosa* increased rapidly in numbers between 15 and 22 August, the worms emerging from the cells in which they had

spent several months in diapause. The decrease in numbers of *A. caliginosa* after 28 August and of *A. nocturna* after 9 September are significant but not due to temperature. The increase of both species after 10 October is significant as is the decline by 2 December. During late summer it was noticed that a high proportion of the cocoons of *A. nocturna* dug out from this field contained worms in a well-advanced stage of development. It would appear that during October cocoons are hatching rapidly and that mortality is high. These two species also show a rapid increase in numbers in April to a level equal to that of the autumn maximum. Part of this increase is due to increased temperature and part to a real increase in population.

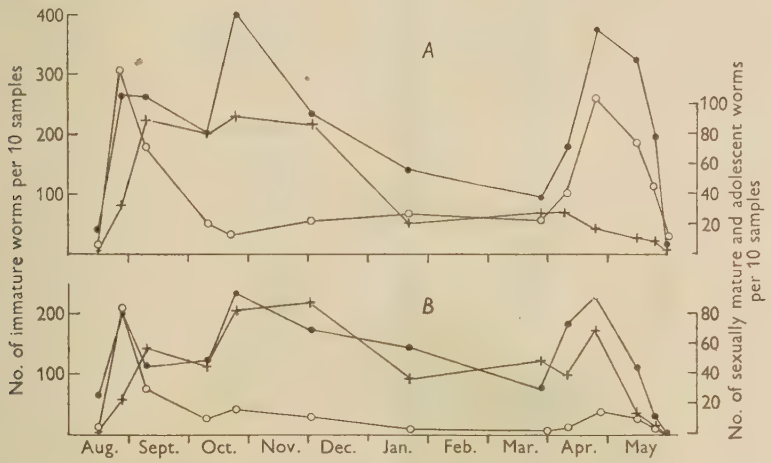

Fig. 2. Seasonal trends in populations of immature, adolescent and sexually mature earthworms. A, *A. nocturna*; B, *A. caliginosa*. ●—●, immature; ○—○, adolescent; +—+, sexually mature.

It is possible to carry the analysis of population changes further for *A. caliginosa* and *A. nocturna* as sufficient individuals of these two species were obtained to classify them into three development groups, immature, sexually mature with well-developed clitellum, and adolescent, i.e. individuals with the tubercles of puberty present and the clitellum delineated but not fully developed. Fig. 2 shows the results.

The initial increase in both species occurs mainly in adolescent and immature worms, and is followed by a rapid drop in the numbers of adolescent and an increase of sexually mature worms, due to further development of some of the adolescent worms. The numbers of sexually mature worms remain constant in *A. nocturna* but show a significant increase for *A. caliginosa* until 2 December. The numbers of adolescent worms decrease until 10 October, remain low during the remainder of the autumn and through the winter, but increase considerably in spring for

A. nocturna and but slightly for *A. caliginosa*. The decrease in the number of sexually mature *A. nocturna* after 2 December is maintained until 16 May, there being no spring increase, and the adolescent and sexually mature worms enter into an obligatory diapause. The winter decrease of sexually mature *A. caliginosa* is followed by an increase in spring, and subsequent reduction when drying soil induces them to enter into a facultative diapause. There is a decline in the number of immatures of both species after the initial maximum is reached at the end of

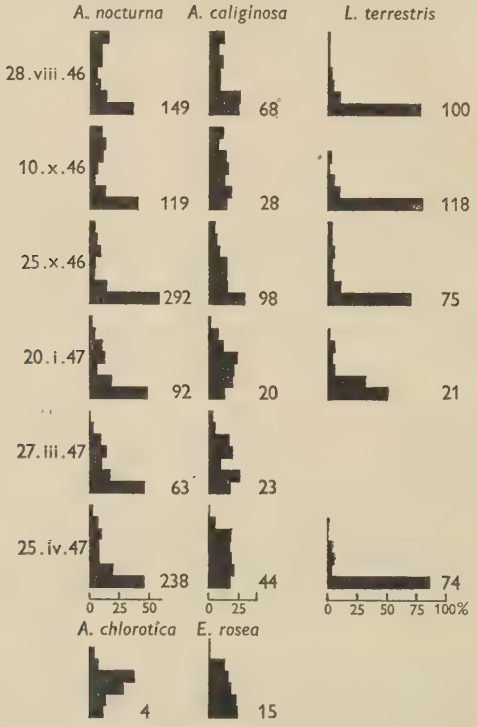

Fig. 3. Distribution of size groups on a percentage basis from 28 August 1946 to 25 April 1947. The number of individuals constituting the lowest weight group is given.

August. Further increase during October is probably due to the emergence of young worms from cocoons. The increase of the total population of *A. nocturna* at this time is due solely to an increase in immature worms; that of *A. caliginosa* to both sexually mature and immature worms. The spring peak of immatures for both species may be partly due to temperature and further emergence from cocoons.

The distribution of the size groups within a population differs considerably between the species at any one time and slightly within the species at different times of the year (see Fig. 3). This distribution was obtained by weighing all individuals at each survey and assigning them to seven uniformly spaced weight

groups depending on the maximum weight of the species. At the survey of 25 April 1947, *Lumbricus terrestris* and *Allolobophora nocturna* showed a predominance of individuals in the lowest weight group, *A. caliginosa* and *Eisenia rosea* showed a more uniform weight distribution, and *Allolobophora chlorotica* a predominance of individuals of medium weight.

Sufficient individuals of *A. cholorotica* and *Eisenia rosea* were not obtained at most surveys to study seasonal changes in size distribution. In *Lumbricus terrestris* and *Allolobophora nocturna* the maximum number of individuals in any one group was always present in the lowest weight group, but this occurred only once for *A. caliginosa.* The proportion of individuals in the lowest weight group of *Lumbricus terrestris* showed a progressive decline from 10 October 1946 to 20 January 1947, suggesting that the rate of emergence was not sufficently rapid to replace the young growing worms. The maximum proportion and absolute number of worms in the smallest weight group for *Allolobophora nocturna* and *A. caliginosa* occurred on 25 October 1946, giving definite evidence that large numbers of young worms were emerging from their cocoons at this time. Since there is but little change in the shape of the distribution diagrams for these two species between 27 March and 25 April 1947 it cannot be decided whether the increased number per sample is due to increase in population and/or increase in temperature.

The striking differences in the shape of the weight distribution diagrams of the three species from 28 August 1946 to 25 April 1947 suggest that the incidence of mortality in the various weight groups differs according to the species. Mortality is relatively greater in the higher weight groups of *Lumbricus terrestris* than of *Allolobophora nocturna*, and of *A. nocturna* than of *A. caliginosa.* The alternative interpretation that mortality is greatest in the lowest weight group in the opposite order of species is less likely since just emerged worms of *A. nocturna* and *Lumbricus terrestris* are similar in size.

Annual changes in population

Sufficient data have not been accumulated to decide whether earthworm populations fluctuate considerably from year to year. Observations in Great Field III are available from September 1945 to May 1947. Great variation is shown at the various sampling dates, but the variation is largely due to soil temperature differences. The correlation coefficient between numbers of worms per sample and soil temperature is highly significant, 0·95, $P < 0{\cdot}01$. Fig. 4 shows the values obtained in the 3 years in relation to the regression, numbers of worms per 10 samples $= 16{\cdot}6 \times$ soil temperature (° F.) $- 383$. On New Zealand it was found that the population was increasing, perhaps as a result of undisturbed soil conditions and improved food supply in the young ley. The increase in the number of worms per ten samples is shown below.

Date	Nos.	Temperature (° F.)
28 March 1946	143	42·3
23 October 1946	246	52·1
10 April 1947	220	40·2

Making allowance for varying temperatures it would appear that there was but little increase in population between 28 March and 23 October 1946 since on Great Field III an increase of temperatures from 42 to 52° F. increased the population by approximately 50%. Between October 1946 and April 1947 there must have been an appreciable increase in population since, in spite of a decrease of 12° F. in temperature, the number of worms per ten samples was only slightly lower. Using the regression data obtained in Great Field III as a guide, the expected number of worms per ten samples would be about 140, whereas 220 were obtained.

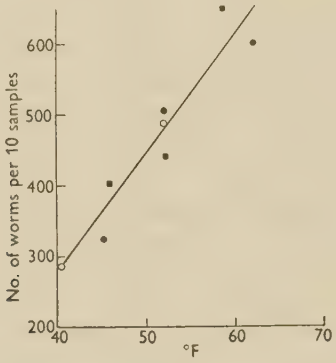

Fig. 4. The effect of temperature on sample numbers from year to year. ●, 1945; ■, 1946; ○, 1947.

All species did not show an increase in population between March 1946 and April 1947, the three large species, *Allolobophora longa*, *A. nocturna* and *Lumbricus terrestris*, showing no advance. The smaller species, *Allolobophora caliginosa*, *A. chlorotica* and *Eisenia rosea*, increased by 100, 50 and 200% respectively. An interesting feature of this population is the marked increase in adults and adolescents between the above sampling dates, varying from two to six times according to the species. Such increases did not occur on two permanent pastures for which data are available on the relevant dates:

	Great Field III		Parklands		New Zealand	
	1946	1947	1946	1947	1946	1947
A. longa	7	4	80	2	0	7
A. nocturna	41	28	50	67	6	11
A. caliginosa	29	37	82	40	6	28
A. chlorotica	4	5	16	12	15	43
E. rosea	5	12	14	22	3	18
L. terrestris	7	3	1	7	2	1

Of the six species, only *Lumbricus terrestris* failed to show a marked increase in the numbers of adults and adolescents per ten samples. It has been shown above

that only a small proportion of the population of this species is present in the higher weight groups and so an increase in the numbers of adults and adolescents could not be expected to occur so soon as in the other species which do not show such a size distribution.

We are indebted to the Agricultural Research Council for a grant to one of us (W. J. McL. G.).

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PERSISTENCE OF D.D.T. AND BENZENE HEXACHLORIDE IN SOILS

By M. S. SMITH
Wye College, University of London

(With 1 Text-figure)

Acid and alkaline soils, both alone and mixed with 2% of D.D.T. or 2% of benzene hexachloride (mixed isomers, containing 10% of the γ-isomer) have been exposed outdoors or subjected to controlled leaching in the laboratory. Residual insecticide has been estimated at intervals by a method involving dehydrohalogenation, and determinations have also been made of chloride content of soil, chloride leached and pH.

Results showed that both D.D.T. and benzene hexachloride were very stable in the soils, about 95% of the former and 80% of the latter being recoverable after 18 months. Very little chloride was leached during this period. The residual insecticide exhibited marked toxicity towards woodlice.

Eighteen months after treatment the benzene hexachloride soils prevented root growth of germinating seeds, while germination and early growth were normal in the D.D.T. soils. This harmful effect of the residual benzene hexachloride was still apparent when 1 part of the treated soil was mixed with 99 parts of the control soil (representing a concentration of less than 0·002% of the γ-isomer).

Introduction

The discoveries within recent years that 2, 2-*bis*(*p*-chlorophenyl)1, 1, 1-trichloroethane (D.D.T.) and benzene hexachloride possess outstanding properties as contact and stomach insecticides, have been followed by intensive research on their chemistry, their mode of action, and their use against specific pests. Apart from their outstanding insecticidal properties, however, these substances owe much of their present importance to the fact that they are stable under ordinary conditions of temperature, light and humidity and so remain as persistent highly insecticidal deposits for considerable periods after application. In view of this persistence and the marked toxicity shown towards most insects, both materials have been examined by a number of workers (e.g. Dawson & Escritt, 1946; Dunn, Henderson & Stapley, 1946; Golightly, 1946; Jameson, Thomas & Woodward, 1947; Jones, 1945; Slade, 1945; Thomas & Jameson, 1946; Wallace, 1946; Wiesmann, 1943; Wiesmann & Fenjves, 1944) as soil insecticides. Such investigations raise the question of the length of time for which these materials will persist in the soil following application. As is well known, the effectiveness of organic compounds such as phenols, cresols, naphthalenes, etc., following soil application is limited by the fact that they undergo fairly rapid decomposition through the agency of micro-organisms. There is already much indirect evidence that both D.D.T. and benzene hexachloride are

moderately persistent in soils, but, as far as the writer is aware, this problem has not been the subject of direct research.

The object of the present investigation was to study the rate of disappearance of benzene hexachloride and D.D.T. in both acid and alkaline soils by estimation of the residual content. Since a likely method of decomposition in both cases is one of dehydrohalogenation, observations were also made on the pH, and the chloride content and chloride leached from the soils were determined at intervals.

Materials and methods

The *soils* used in these investigations were:

I. Brickearth, very fine sandy loam, from newly ploughed pasture, pH 5·9. Described below as 'acid soil'.

II. Brickearth, very fine sandy loam, from temporary ley, pH 7·8. Described below as 'alkaline soil'.

Soils I and II were used in the outdoor experiments.

III. Sandy loam from pasture on Folkestone Sand, pH 5·8.

Soil III was used in the laboratory leaching experiments and is described below as 'leached acid soil'.

Before use the soils were air dried and passed through a 2 mm. sieve.

D.D.T. Pure, twice recrystallized, *p*, *p*′-D.D.T., m.p. 107° C. was used throughout.

Benzene hexachloride. Pure samples of α-, β-, γ- and δ-isomers were used in the preliminary experiments and a commercial mixture containing 10% by weight of the γ-isomer was used in the soil experiments.

α-isomer. Fine white plate-like crystals, m.p. 156–158° C., softens at 152° C.

β-isomer. Fine white crystalline powder, m.p. above 295° C.

γ-isomer. Coarse white crystals, m.p. 111–113° C., softens at 107° C.

δ-isomer. Fine white crystalline powder, m.p. 139–140° C.

Mixed isomers. Fine buff-coloured powder containing a few coarse granules, m.p. 95–145° C., softens at 72° C.

Incorporation of insecticide with soil

For the outdoor experiments, 50 g. of the insecticide were well ground in a mortar with 500 g. of soil. This mixture was then divided into two equal parts and each was further ground with 1 kg. of soil. Taking a series of equal volumes from each portion in turn, the two parts were well mixed together in a large glass trough. For the laboratory experiments, 10 g. of insecticide were well ground in a mortar with 500 g. of soil. It was hoped that this method would lead to a homogeneous mixture but fluctuations in the results suggest that this may not have been completely attained. Although this concentration (2%) was much greater than any likely to be used in practice, it was more suitable from the analytical standpoint.

Outdoor experiments

The acid and alkaline brickearths were placed in inverted Winchester bottles, the bottoms of which had been cut off, and in 6 in. funnels. Each Winchester bottle contained 2 kg. of soil and each funnel 100 g. Vessels were placed to collect drainage. At intervals, samples of the soils were taken for determination of (i) D.D.T. or benzene hexachloride content, (ii) chloride content, (iii) pH. Soil chloride was determined on the soils in the funnels only, the whole of the contents of one funnel being taken and thoroughly mixed. Chloride was also determined in the leachings from both Winchester bottles and funnels.

Laboratory experiments

50 g. portions of the 'leached acid soil' were placed in a series of eight 6 × 1 in. boiling tubes with a hole in the base to provide drainage outlet. The soils were leached with distilled water at intervals and the drainage collected. After measured times, one tube from each set was emptied and determinations made on the soil content as above. Chloride was also determined on the combined leaching from each set of tubes.

Biological tests

Bacterial counts. A bacterial count was made by the plate method on the soils used in the outdoor experiments after 11 months. An agar-egg albumen medium was used and three plates were poured from each of the dilutions 10,000, 100,000 and 1,000,000. The incubation temperature was 31° F.

Tests on germination and seedling growth. The soils were placed in earthenware dishes and seeds of various crops were sown and their germination and early growth observed. Treated soils mixed with ninety-nine times their weight of untreated soil were also used.

Insecticidal action. 15 g. of the moist soil under test were placed in each of three Petri dishes, twenty woodlice added to each and daily counts taken.

Experimental

Estimation of D.D.T. and benzene hexachloride

The method adopted involved dehydrohalogenation with alcoholic potash followed by titration of the excess alkali with standard acid (e.g. Soloway, Schechter & Jones, 1946).

(*a*) *D.D.T.*

Although D.D.T. is known to liberate more than one equivalent of HCl when boiled under reflux with alcoholic potash (Wain & Martin, 1947), under the comparatively mild conditions of dehydrohalogenation used here (0·05 N alkali, 15 min. boiling) the value was found to differ little from unity (1·001, 1·007, 0·988, 1·001, 0·998).

Known weights (about 200 mg.) of D.D.T. were well mixed with soil (10 g.) and

Soxhlet extracted with light petroleum for 4 hr. After removal of solvent, the residue was dissolved in 25 ml. alcohol and 25 ml. 0·1 N alcoholic potash added. The solution was then boiled under reflux for 15 min., cooled and excess alkali determined by titration with standard acid using phenolphthalein as indicator (Table 1).

TABLE 1. *Estimation of D.D.T. in prepared soil mixtures*

Wt. of D.D.T. taken (mg.)	Wt. of D.D.T. found (mg.)	Recovery (%)
204·1	199·5	97·8
186·6	185·2	99·3
233	226·7	97·3

The above method was adopted for the estimation of D.D.T. in the soil experiments. A blank experiment carried out on the untreated soil gave a small correction which was applied in all determinations.

(*b*) *Benzene hexachloride.*

Dehydrohalogenation at room temperature. Standard solutions of the separate and mixed isomers were prepared containing, where solubility permitted, about 1 g. in 250 ml. of absolute alcohol. 25 ml. quantities of these were treated with 25 ml. 0·1 N alcoholic potash and allowed to stand at room temperature. After timed intervals, the excess alkali was titrated against standard acid. The results are plotted in Fig. 1.

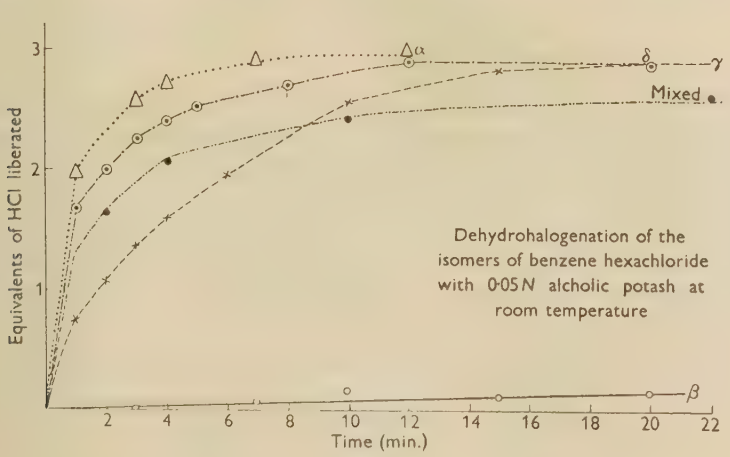

Fig. 1

Dehydrohalogenation under reflux. In each case the dehydrohalogenation was examined after boiling under reflux for (*a*) 15 min., (*b*) 30 min. Only in the case of the β-isomer was the previous limiting value of equivalents of HCl liberated appreciably affected (Table 2).

TABLE 2. *Equivalents of* HCl *liberated from benzene hexachloride on boiling with* 0·05N *alcoholic potash*

Time refluxed (min.)	Isomer				
	α	β	γ	δ	Mixed
15	2·88	2·48	2·88	2·90	2·75
30	2·92	2·84	2·91	2·93	2·84

Dehydrohalogenation under reflux was adopted in all subsequent estimations.

Determination of factor for mixed isomers. It was first necessary to determine a 'factor' representing the number of equivalents of hydrogen chloride liberated when the mixed isomers were Soxhlet extracted with light petroleum and the soluble material, after removal of solvent, boiled with 0·05N alcoholic potash for 15 min. as above. The results obtained are shown in Table 3.

TABLE 3. *Equivalents of* HCl *liberated by mixed isomers after extraction with light petroleum*

Solvent	
Rectified spirit	Absolute alcohol
2·73	2·62
2·62	2·61
2·55	2·68
2·63	

The variation in the results was outside the limit of experimental error, suggesting that the mixture was not homogeneous. The average value of 2·63 equivalents of HCl liberated was used as the 'factor' in subsequent estimations.

The results of trial estimations on prepared mixtures of weighed amounts (about 200 mg.) of the mixed isomers well ground with 10 g. of soil are given in Table 4.

This method was adopted for the estimation of benzene hexachloride in the soil experiments. A blank estimation on the control soil provided a small correction.

TABLE 4. *Estimation of benzene hexachloride in prepared soil mixtures*

Wt. of benzene hexachloride taken (mg.)	Wt. of benzene hexachloride estimated (mg.)	Recovery (%)
179·4	182·8	101·9
235·5	231·4	98·2
223·6	224·0	100·2
236·6	238·1	100·7
264·2	262·5	99·4
285·2	283·9	99·5
195·4	196·5	100·6

Chloride content of soil

40 g. of air-dry soil were shaken mechanically with 200 ml. of water for an hour (with the leached acid soil, 20 g. of soil and 150 ml. of water were used), left to settle overnight and filtered. 50 ml. of the filtrate were diluted to 100 ml., 1 ml. 5% potassium chromate added and the solution titrated against 0·01 N silver nitrate in a porcelain dish. A blank estimation on 100 ml. distilled water was run to the same tint and subtracted from the titre.

Chloride in leachings. 100 ml. of the leachings were titrated against 0·01 N silver nitrate as above.

pH *of soil.* Determined on the air-dry soil by the glass electrode.

Results

Table 5 shows the D.D.T. and benzene hexachloride content of the soils on various dates, Table 6 the chloride content of the soils and Table 7 the chloride content of the drainage water.

Many observations of pH were made, but since no clear conclusions could be drawn from them, the ranges and means only of these are recorded in Table 8.

Table 5. *Percentage residual D.D.T. and benzene hexachloride content of soils (average of duplicates)*

	Acid soil		Alkaline soil	
Date	D.D.T.	Benzene hexachloride	D.D.T.	Benzene hexachloride
	Outdoor experiments			
Soils in inverted Winchester bottles:				
23. v. 46	2·05	1·98	1·93	1·87
18. vi. 46	1·90	1·76	1·91	1·77
9. vii. 46	1·86	1·77	1·93	1·70
29. vii. 46	1·82	1·69	1·84	1·66
6. ix. 46	1·87	1·64	1·90	1·74
6. xi. 46	1·95	1·77	2·00	1·77
8. i. 47	1·97	1·74	1·98	1·88
10. iii. 47	2·11	1·75	1·94	1·91
16. iv. 47	1·89	1·87	1·97	1·77
28. vi. 47	1·97	1·68	1·96	1·77
27. viii. 47	1·94	1·61	1·87	1·73
3. x. 47	1·95	1·74	1·93	1·75
Soils in funnels:				
23. v. 46	2·05	1·98	1·93	1·87
29. vii. 46	1·87	1·64	1·90	1·72
16. xii. 46	1·75	1·57	1·97	1·62
21. xi. 47	1.92	1·49	1·68	1·35
	Laboratory experiment (leached acid soil)			
1. x. 46	1·88	1·99	—	—
8. i. 47	1·88	1·86	—	—
15. ii. 47	1·95	1·83	—	—
26. iii. 47	1·83	1·79	—	—
3. vi. 47	1·93	1·88	—	—
12. vii. 47	1·92	1·85	—	—
8. ix. 47	1·88	1·88	—	—
19. xi. 47	1·90	1·71	—	—

TABLE 6. *Chloride content of soils* (mg./100 g.)

Date	Acid soil			Alkaline soil		
	Control	D.D.T.	Benzene hexachloride	Control	D.D.T.	Benzene hexachloride
			Outdoor experiment			
23. v. 46	7·4	7·7	11·7	5·2	5·4	11·5
29. vii. 46	1·1	0·5	5·8	0·1	1·5	2·0
21. xii. 46	3·5	0·5	4·9	1·6	5·6	1·8
21. xi. 47	3·9	3·3	5·5	2·9	3·0	2·8
			Laboratory experiment			
1. x. 46	18·0	18·0	22·5	—	—	—
8. i. 47	3·8	0·1	7·9	—	—	—
15. ii. 47	0·3	3·6	0·3	—	—	—
26. iii. 47	Nil	1·8	7·6	—	—	—
3. vi. 47	0·6	0·5	1·1	—	—	—
12. vii. 47	4·1	3·9	2·6	—	—	—
8. ix. 47	Nil	2·8	1·7	—	—	—
19. xi. 47	Nil	3·0	0·2	—	—	—

TABLE 7. *Chloride content of drainage water*

Period of collection	Acid soil			Alkaline soil		
	Control	D.D.T.	Benzene hexachloride	Control	D.D.T.	Benzene hexachloride
			Outdoor experiments			
Soils in inverted Winchester bottles (mg.):						
23. v.–6. ix. 46	9·4	6·2	58·8	12·7	20·5	56·1
26. iii.–28. vi. 47	15·0	19·0	8·4	9·9	8·1	12·5
28. vi.–21. xi. 47	8·0	4·7	6·5	3·7	6·0	27·4
Soils in funnels (mg./100 g.):						
23. v.–16. vii. 46	9·8	10·5	16·7	7·3	7·9	25·9
16. vii.–12. ix. 46	5·2	5·0	6·4	5·7	5·1	8·8
26. iii.–19. v. 47	4·5	2·0	1·2	3·1	2·0	4·0
19. v.–28. vi. 47	2·9	3·0	2·2	1·3	4·0	2·4
28. vi.–21. xi. 47	5·9	7·4	10·1	6·1	7·9	10·9
			Laboratory experiment (leached acid soil, mg./100 g.)			
Leaching period:*						
1	15·7	15·9	15·8	—	—	—
2	0·7	0·3	2·7	—	—	—
3	0·1	Nil	0·8	—	—	—
4	1·3	1·3	4·2	—	—	—
5	Nil	Nil	1·9	—	—	—
6	0·2	0·1	1·5	—	—	—
7	0·5	0·7	2·1	—	—	—
Total chloride leached/100 g. soil	18·5	18·3	29·0	—	—	—

* Leaching periods consecutive and each of 6–8 weeks.

TABLE 8. pH *values of soils—ranges and means*

	Control	D.D.T.	Benzene hexachloride
	Acid soil		
Range of pH	5·8–6·9	5·7–6·3	5·9–6·6
Mean of all observations	6·07	5·94	6·23
	Alkaline soil		
Range of pH	7·1–8·0	7·2–8·0	7·2–8·1
Mean of all observations	7·64	7·73	7·62
	Leached acid soil		
Range of pH	5·8–6·6	5·8–6·7	5·8–6·7
Mean of all observations	6·26	6·30	6·27

Bacterial numbers in the soils in the outdoor experiments were estimated in April 1947 (a late spring), and are recorded in Table 9.

TABLE 9. *Millions of bacteria per g. of air-dry soil*

Acid soil	Control	1·47
	D.D.T.	4·86
	Benzene hexachloride	3·95
Alkaline soil	Control	2·75
	D.D.T.	2·96
	Benzene hexachloride	4·17

Germination and seedling growth

Five months after the commencement of the outdoor experiments, samples of the soils were taken to determine whether the residual insecticide had any harmful effect upon germination and early growth. Using cress seeds, it was found that no retardation occurred with D.D.T., but with benzene hexachloride germination was slower than in the controls, the cotyledons did not expand and shed the seed coat, the hypercotyl was much curved, the root showed brown patches, no root hairs were visible and the root did not strike down into the soil. Microscopic examination showed furrowing and jags at the edge of the root and the normal rounded curvature of the tip was replaced by a sharp angular shape. Experiments conducted 7 months later confirmed the earlier results with cress, and showed a similar effect with oats; controls and D.D.T.-treated soils allowed normal germination and seedling growth, but benzene hexachloride did not permit root development and top growth remained very stunted.

Since the concentration of insecticide was much in excess of that likely to be used in practice, experiments were made to reveal the lowest concentration at which root development was affected. Oats were found to be particularly susceptible to benzene hexachloride and a concentration of 0·0025% was sufficient to influence root development. At a concentration of 0·025% no development of fibrous root

occurred, and only a few short adventitious roots appeared. Root development of cress was slightly affected at 0·025% and more so at 0·05%.

After 18 months this harmful effect was still apparent in the benzene hexachloride-treated soils when sown with wheat, cabbage, sugar beet or flax. Reduction of the concentration of insecticide to approximately 0·02% by admixture with the original soil led to a similar effect on wheat to that previously observed with oats; a few short brownish adventitious roots only appeared. Root growth of cabbage, sugar beet and flax was affected to a lesser extent. Seedlings in the D.D.T.-treated soils developed normally, although the percentage germinating was reduced somewhat in the case of cabbage and flax.

Insecticidal action

The results of insecticidal tests of the D.D.T. and benzene hexachloride soils after 18 months towards *Oniscus ascellus* are assembled in Table 10. Results are also shown for the benzene hexachloride-treated soils when 1 part of these was mixed with 99 parts of the original soil, so reducing the concentration of insecticide to about 0·02%.

TABLE 10. *Insecticidal action of treated soils towards* Oniscus ascellus

		% Mortality after 96 hr. (average of triplicates)
Acid soil	Control	0
	D.D.T.	73
	Benzene hexachloride	100
Alkaline soil	Control	0
	D.D.T.	78
	Benzene hexachloride	100
Acid soil treated benzene hexachloride mixed with 99 parts original soil		67
Alkaline soil treated benzene hexachloride mixed with 99 parts original soil		88

DISCUSSION

The results recorded in Tables 1 and 4 demonstrate that D.D.T. and benzene hexachloride can be estimated in soil, with an accuracy of about 3%, by extraction with light petroleum followed by dehydrohalogenation with alcoholic alkali. Under the conditions used, D.D.T. liberated almost exactly one, and the specimen of mixed isomers of benzene hexachloride 2·63 equivalents of hydrogen chloride, although the pure individual isomers approached 3 (Tables 2, 3 and Fig. 1).

That both D.D.T. and benzene hexachloride are markedly persistent in soils when applied in large concentrations is clearly demonstrated by the results of Table 5. During the period of these experiments the soils were exposed to varying weather conditions which included a cold wet summer, a very cold winter and a hot

dry summer. The same persistence was shown in the acid soil subjected to controlled leaching in the laboratory. The small fluctuations in the content of insecticide may be attributed in part to the initial difficulty in obtaining a homogeneous mixture with the soil. The lower benzene hexachloride content of the soils exposed in funnels compared with the same soils in inverted Winchester bottles might be explained by a greater volatilization in the former case, where there is a large surface area and a small bulk of soil. About 95% of the D.D.T. and 80% of the benzene hexachloride were recovered from the soils kept in the open after 18 months and whether the soil was acid or alkaline was apparently immaterial.

The chloride content of the soils (Table 6) remained low throughout, and only in the case of the benzene hexachloride-treated soils were the values normally above the controls. This was partly due, especially in the early estimations, to the small water soluble chloride content of the mixed isomers (0·066%). This factor is also apparent in the early results recorded in Table 7, where the chloride leached from the benzene hexachloride-treated soils was initially in excess of that from the control. Subsequently the difference became less marked. The chloride leached from the D.D.T.-treated soils was not significantly different from the controls. From the results with the leached acid soil (where the drainage was collected throughout the whole run of the experiment) the excess of total chloride leached from the benzene hexachloride-treated soil above that of the control was only 10·5 mg./100 g. of soil. The insecticide present, on complete decomposition, was capable of yielding 1450 mg. of chloride.

The pH values of the soils (Table 8) showed no regular variation or trend towards increasing acidity and are, therefore, in agreement with the results discussed above.

The bacterial numbers (Table 9) do not indicate any harmful effect of the insecticides upon these organisms. Moreover, the treated soils did not show the marked rise in bacterial numbers which would have been expected if the added materials were being utilized as sources of energy (Matthews, 1924).

That D.D.T. and benzene hexachloride still maintain their insecticidal activity after 18 months in the soil was shown by the experiments on woodlice (Table 10).

The residual benzene hexachloride was found to exert a harmful effect on the growth of seedlings, especially to their root development. This effect was still apparent when the insecticide concentration was reduced to about 0·02% by mixing with the control soil. This concentration, representing 0·002% of the γ isomer is equivalent to an application of about 15 lb. of the γ isomer per acre when incorporated with the top 3 in. of soil, or some twenty times the amount of a dressing considered adequate for pest control (Jameson *et al.* 1947). Consequently, although plant damage need not be expected to arise in practice from soil application of benzene hexachloride, high local concentrations might prove dangerous. No harmful effect was observed in the case of the D.D.T.-treated soils and it may be concluded that there is a wide safety range in the use of this substance for soil application.

The writer is grateful to Dr R. L. Wain for suggesting the present study and for interest and advice throughout. She also desires to thank Imperial Chemical Industries, Ltd., for specimens of benzene hexachloride, pure and mixed isomers, and Mr W. J. Ball for carrying out pH determinations.

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THE CONTACT TOXICITY OF A NUMBER OF D.D.T. ANALOGUES AND OF FOUR ISOMERS OF BENZENE HEXACHLORIDE TO *MACROSIPHONIELLA SANBORNI* AND *ORYZAEPHILUS SURINAMENSIS**

By K. A. LORD
Rothamsted Experimental Station, Harpenden, Herts

(With 7 Text-figures)

Twenty-three analogues of D.D.T. and four isomers of benzene hexachloride have been compared with D.D.T. for toxicity as contact insecticides using a direct spraying technique. Each substance has been tested against two insect species: *Macrosiphoniella sanborni* and *Oryzaephilus surinamensis*. The substances tested included a number of halogen and alkyloxy analogues of D.D.T. and a number of compounds of varying degrees of chlorination between diphenylethane and D.D.T.

The biological data have been examined, as far as possible, by the method of probits.

The probit lines for the D.D.T. analogues varied both in position and in slope: those for the isomers of benzene hexachloride in position but not in slope. In general, neither the relative positions nor the relative slopes of the probit lines for the various substances were the same against the two insect species.

The toxicity of substances analogous to D.D.T. appears to be related to molecular weight, with maximum toxicity occurring in the molecular weight range 300–450.

Among D.D.T. analogues the slope of the probit line is apparently correlated with molecular volume. The variation of the slope of the probit lines has been discussed, and it has been concluded that it arises from differences in the interaction of the poisons with the test-subjects.

The various theories relating to the toxicity of D.D.T. and B.H.C. have been discussed in relation to the experimental data described here. To account for all the experimental results by means of one theory it is necessary to postulate a physico-chemical mechanism of toxic action. Such a mechanism implies that the intensity of biological action will be dependent upon the physico-chemical properties of the poisons, whether or not a specific molecular configuration is involved, and toxicity is therefore dependent on the molecule as a whole.

Introduction

The introduction of the insecticide D.D.T. (2, 2-bis (*p*-chlorphenyl), 1, 1, 1-trichloroethane) to the United Kingdom came at a time when the war was increasing the demands for insecticides and at the same time bringing about the loss of important sources of supply. The immediate importance of D.D.T. was for control of disease-spreading insects at the battle fronts, but the possible value of the insecticide in the agricultural field was not lost sight of. The persistence and stability of D.D.T., properties previously unknown in an organic contact insecticide, open up new possibilities in plant protection, but the biological properties of D.D.T. are not ideal: it is often slow-acting and it is not equally toxic to all species of arthropods.

* Part of a thesis approved by the University of London for the degree of Ph.D.

Indeed, in some cases it may lead to a marked increase in some pests (e.g. red spider) probably through its effect upon their parasites. An insecticide possessing the persistence of D.D.T. with a different specificity range and a higher speed of toxicity to insects would be very useful. It is desirable on these and on other grounds to synthesize and test the compounds of a similar structure to D.D.T., since it is likely that changes in the molecule will affect the relative as well as the absolute potency of the compound. Work on these lines has been carried out by a number of investigators: Martin & Wain (1944*b*), Siegler & Gertler (1944), Prill, Hartzell & Arthur (1945), Prill, Synerholm & Hartzell (1946), Busvine (1946), Proverbs & Morrison (1947) and Ginsburg (1947). Much of it has been carried out contemporaneously with the work to be described and although the results of these other workers have now been published, they were not available when this work was started. For this type of work it is desirable that the latest biological and statistical methods should be employed in order to obtain as high a precision as possible, so that the effects of chemical constitution and substitution, as well as physical and chemical properties on the toxicity of this group of insecticides, may be determined.

Test-subjects and technique of biological assay

Because such work required extensive biological trials it was essential that the insect test-subjects selected should be readily available in large numbers. If such tests are to be strictly comparable it is also preferable that the same method of testing be employed in each case. A further requirement is that the test-subjects should be fairly susceptible to the poison used as a starting-point so that less toxic substances may also be conveniently tested. These limitations, together with those imposed by the technique employed for biological testing, considerably restrict the range of possible insect test-subjects. The insects used in this investigation were the aphid *Macrosiphoniella sanborni* and the saw-toothed grain beetle *Oryzaephilus surinamensis*, widely differing types which also satisfy all the conditions set out above.

The substances were tested for their contact action only, using a direct spraying technique. Throughout the experiments D.D.T. was included as a standard with which to compare the toxicity of substances tested on different occasions, and to provide a check on effects caused by variations in the resistance of the test-subjects. Since atmospheric environment, especially after treatment (Potter & Gillham, 1946), is liable to affect toxicity, the conditions after spraying were standardized as far as facilities permitted, and details of atmospheric conditions are given with each test.

Medium

The effect of media on the toxicity of insecticides is known to be great (Martin, 1943; Tattersfield, Potter & Gillham, 1947). Therefore, when making comparisons of the toxicity of a number of compounds, in order to avoid the introduction of

unknown factors, it is essential that the same medium be used in each case. Moreover, it is essential that the poison in each case be in a comparable state of division, since when a contact insecticide is applied as a suspension its toxicity is influenced to a considerable extent by the particle size and shape of the poison (McIntosh, 1946).

The use of emulsions of an organic solvent immiscible with water as media for testing the poisons, avoids the difficulties arising in the preparation of suspensions of uniform particle size, and this type of medium was therefore employed.

Benzene is a good solvent for D.D.T. and its analogues, and since a 10% emulsion of benzene in water, with 0·05% cyclohexylamine dodecyl sulphate as emulsifying agent, is not toxic to the test-subjects selected, this medium was used throughout the tests.

Treatment of results

Where suitable data were obtained from the toxicological trials they have been examined by the method of probits (Bliss, 1935), and, where necessary, adjustments for mortality in the controls have been made (Finney, 1944).

The (molecular concentration) $\times 10^6$ required to give 50% kill for each substance has been calculated from the equation to the fitted probit regression lines. Where parallel determinations have been made, weighted means have been obtained (Table 3). The slopes of probit lines have been listed (Table 4) and weighted means obtained where parallel biological trials were carried out.

These two values are sufficient to describe any probit line. Comparison of the probit lines is difficult when the data are given in this form, therefore the concentrations of poisons giving 25, 50, 75 and 95% kill have been calculated from the probit regression lines. Where parallel determinations were made, weighted mean values only were used; these figures recalculated to give relative potencies (D.D.T. = 1000) are set out in Table 5.

Preparation of symmetrically p*-substituted halogen analogues of D.D.T.*

The preparation of D.D.T. is described by Zeidler (1874) in the first section of the symposium 'Untersuchungen über die synthetische Darstellung von aromatischen Verbindungen durch Wasserentziehung'. It is obvious from the symposium that a general reaction occurs between the aldehydes and aromatic compounds. First mention of the reaction appears to have been made by Baeyer (1872). Later workers have extended the examples of reaction between aromatic compounds and the carbonyl groups. A few examples are given here, but an exhaustive review is not intended. Chattaway (1926) prepared numbers of derivatives from chloral and substituted phenols using the original catalyst sulphuric acid. Harris & Frankfurter (1926) prepared similar derivatives using anhydrous aluminium chloride as catalyst. Baekland & Bender (1925) used hydrochloric acid. The same reaction has been extended to ketones and aromatic compounds, e.g. the condensation of two molecules of phenol with one molecule of cyclohexanone (Carpmael, 1928).

The reaction takes place especially between benzene rings substituted with *o-p*-directing groups and aldehydes. If the position *para* to the *o-p*-directing group is free, the main product of reaction is the *para*-substituted derivative, otherwise substitution occurs in the *o*-position (Chattaway, 1926).

Using this method and sulphuric acid as catalyst it was found that the preparation of a number of *pp'*-halogen analogues of D.D.T. could be readily carried out. All but one of the compounds listed in Table 1 were prepared in this way.

Experimental

Preparation of D.D.T. analogues

In preparing the symmetrically substituted halogen analogues of D.D.T., the method employed by Zeidler (1874) was found to be most convenient. The experimental details were as follows:

A mixture of one molecule of chloral hydrate with two molecules of monohalogen benzene was treated with five times its own volume of concentrated sulphuric acid. The mixture was heated for a few minutes on a water-bath and was then well stirred until it was cold. The mixture was poured on to ice and the solids separating filtered off and crystallized from alcohol.

A similar series of products was obtained when bromal was used instead of chloral hydrate. The yields in these cases were, however, poor.

An oil separated when the reaction mixture of fluorbenzene and chloral hydrate and sulphuric acid was poured on to ice. It was extracted with ether. The ether solution was washed well with water and the ether was evaporated off. The oil separating was taken up in alcohol from which a crop of white crystalline material was eventually obtained after prolonged cooling.

Preparation of (p-*chlorphenyl*) 1-*phenyl*-2, 2, 2-*trichlorethane*

Trichlormethyl phenyl carbinol was prepared from benzaldehyde and chloroform as described by Jocicz (1897). The phenyl trichlorocarbinol was then condensed with chlorobenzene to give the desired product. A well-stirred equimolecular mixture of phenyl trichlorocarbinol and chlorobenzene was treated with excess sulphuric acid. A rapid reaction took place. The mixture was allowed to stand for some time. It was then poured on to ice. An oil separated which was induced to crystallize by scratching. The solid was filtered off and recrystallized from alcohol.

1, 1-*bis* (p-*chlorphenyl*) *ethane and diphenyl ethane*

These compounds were prepared by condensing chlorobenzene and benzene respectively with acetaldehyde using aluminium chloride as catalyst (Bodendorf, 1931).

$(ClC_6H_4)_2CH.CH_3$	B.p. 194–196° C.	14 mm. Hg
$(C_6H_5)_2CH.CH_3$	B.p. 144° C.	14 mm. Hg

Confirmation of structure of D.D.T. analogues

The removal of one molecule of HCl by alkali from D.D.T. takes place quantitatively. The ethylenic derivative so obtained may be readily oxidized by means of chromic acid to the corresponding benzophenone derivative (Harris & Frankfurter, 1926):

$$(ClC_6H_4)_2CH.CCl_3 + NaOH \rightarrow (ClC_6H_4)_2C{=}CCl_2 + NaCl$$

$$(ClC_6H_4)_2C{=}CCl_2 \xrightarrow{CrO_3} (ClC_6H_4)_2C{=}O.$$

The reactions were, therefore, used in order to check the purity and constitution of the D.D.T. analogues prepared. The melting-points of the benzophenone obtained from the various D.D.T. analogues are listed in Table 1. Also included in the table are the numbers of atoms of chlorine or bromine removed from one molecule of D.D.T. analogue by the action of caustic soda.

TABLE 1. *Decomposition of D.D.T. analogues*

D.D.T. analogue	M.p. (° C.)	M.p. of benzophenone derivative (° C.)	No. of atoms of halogen removed by NaOH from one molecule of analogue
$(FC_6H_4)_2CH.CCl_3$	43–45	106–107	0·99
$(ClC_6H_4)_2CH.CCl_3$	109	146–147	1·00
$(BrC_6H_4)_2CH.CCl_3$	141–144	175–175·5	1·00
$(IC_6H_4)_2CH.CCl_3$	177–179	236–237	1·00
$ClC_6H_4.C(C_6H_5)H.CCl_3$	76·5–77·5	75–77	1·00
$(C_6H_5)_2CH.CCl_3$	62·5–64	—	—
$(FC_6H_4)_2CH.CBr_3$	73·4–74	105–106·5	1·46
$(ClC_6H_4)_2CH.CBr_3$	144–145	147–148	1·23
$(BrC_6H_4)_2CH.CBr_3$	171–172·5	176–176·5	1·11
$(IC_6H_4)_2CH.CBr_3$	190–191	237–238	1·13

It is obvious from the reaction, that one molecule of hydrochloric acid is removed quantitatively from the derivatives of diphenyltrichloroethane. The reaction does not appear to be quantitative for the derivatives of diphenyl tribromoethane. It was, however, noticed that decomposition of tribromoethane analogues occurred during crystallization; thus it was not possible to purify them beyond a certain limit, and the presence of impurities may account for the apparent removal by caustic soda of more than one atom of bromine per molecule.

A further list of substances tested is to be found in Table 2.

TABLE 2*

	M.p. (° C.)
$(Cl_6H_4)_2CH.CHCl_2$	110
$(CH_3OC_6H_4)_2CH.CCl_3$	88
$(CH_3.CH_2.OC_6H_4)_2CH.CCl_3$	107
$(CH_3.CH_2.CH_2.OC_6H_4)_2CH.CCi_3$	62
$(CH_3.CH_2.CH_2.CH_2.OC_6H_4)_2CH.CCl_3$	52
$(CH_3.CH_2.CH_2.CH_2.CH_2.OC_6H_4)_2CH.CCl_3$	66

* The substances listed in this table were kindly presented by the Ministry of Supply. All are *p*, *p'*-substituted derivatives of 1, 1-diphenyl-2, 2, 2-trichlorethane.

Insect test-subjects

Macrosiphoniella sanborni Gill. Chrysanthemum aphid. The insects were reared on chrysanthemum plants in a glasshouse. No form of caging was employed, the colonies being maintained on plants standing on the glasshouse staging. No attempt was made to control either temperature or humidity. Only adult apterous viviparous parthenogenetic females were used for test purposes.

Oryzaephilus surinamensis L. The insects were reared on rolled oats in glass jars, closed with muslin in a constant temperature cabinet at approximately 35° C. The atmosphere of the cabinet was maintained in a humid condition by means of pads of cotton wool soaked in water. The rolled oats were fumigated with carbon disulphide before they were used to prepare a culture, which was made up by half filling a 2 lb. jam jar with rolled oats to which 100 adult insects were added. Their progeny, when adults, were used for test purposes.

Methods and apparatus

The various substances were tested for insecticidal activity as benzene solutions emulsified in water, using a direct spraying technique (Tattersfield & Potter, 1943). The apparatus which was used for the application of the preparations as sprays was that described by Potter. Throughout the experiments a gap of 1·7 in. was used, and the pressure at which the spray was atomized was adjusted to give an approximately constant weight of deposit per unit area. 5 ml. aliquots of spray fluid were used in all cases.

The insects were sprayed in Petri dishes of 9 cm. diameter. The bottoms of the dishes were covered with circles of tricoline. After treatment the tops of the dishes were covered with a piece of muslin held in place by a rubber band. The insects were then removed to a storage room until an inspection was made.

Treatment of insects before spraying

Macrosiphoniella sanborni. On the morning of the day of spraying the insects were removed from the host plant by a few sharp taps, and those required were selected and counted into 2 × 1 in. glass tubes using a camel-hair paint brush. The tubes were closed with pieces of muslin held in position by rubber bands and the insects kept at room temperature until they were sprayed.

Oryzaephilus surinamensis. The insects were removed from culture on the morning of the day of spraying and were counted into 2 × 1 in. glass tubes using a camel-hair paint brush. The tubes were closed with muslin held in position by rubber bands. For about an hour before spraying commenced, the insects were maintained at 60–65° F. This treatment was employed because the insects are more readily handled after exposure to this lower temperature.

Treatment of insects after spraying

Neither type of test-subject was fed after spraying. The *M. sanborni* were inspected on the day after they were sprayed, and the *O. surinamensis* 3 days after spraying.

Examination of insects

The insects were inspected using a warm plate at 37–40° C. (Tattersfield & Potter, 1943). The insects were classified in the following way: *N*, normal insects; *S*, slightly affected insects which are able to progress but which drag a leg; *B*, badly affected insects able to progress but which are obviously paralysed and unable to progress in an orderly fashion; *M*, moribund insects not dead but unable to progress; *D*, dead insects showing no signs of life.

Results of biological assay

Full experimental results are available at Rothamsted. A summary is given here in Tables 3–6. The general types of result obtained are illustrated by Figs. 1–4.

In comparing the toxicity of the compounds tested in the experiments described here, account must be taken of the fact that the probit regression lines of the substances tested are not parallel. Large statistically significant differences in the slopes of the probit lines have been observed. In a number of cases the determinations were repeated and the differences confirmed. There are, therefore, no grounds for supposing that the differences in slope are due to chance effects. Therefore, in no cases have lines obtained using different poisons been constrained to parallelism (even where the differences in slope are not significant) and account has been taken, as far as possible, of the differences of relative toxicity at various mortality levels.

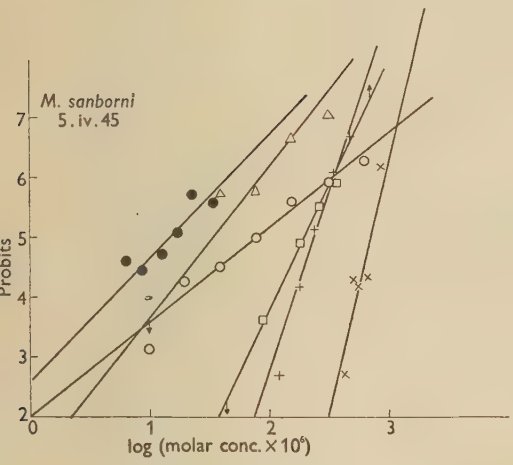

Fig. 1

● $(ClC_6H_4)_2CH.CCl_3$ ○ $(ClC_6H_4)_2CH.CHCl_2$
△ $ClC_6H_4.C(C_6H_5)H.CCl_3$ + $(ClC_6H_4)_2CH.CH_3$
□ $(C_6H_5)_2CH.CCl_3$ × $(C_6H_5)_2CH.CH_3$
↑ 100 % mortality ↓ 0 % mortality

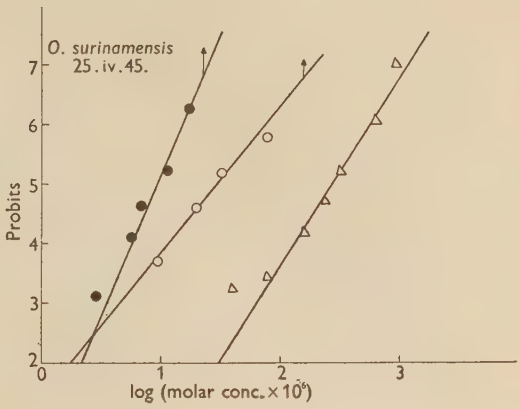

Fig. 2

● $(ClC_6H_4)_2CH.CCl_3$ ○ $(ClC_6H_4)_2CH.CHCl_2$
△ $ClC_6H_4.C(C_6H_4)H.CCl_3$ ↑ 100 % mortality

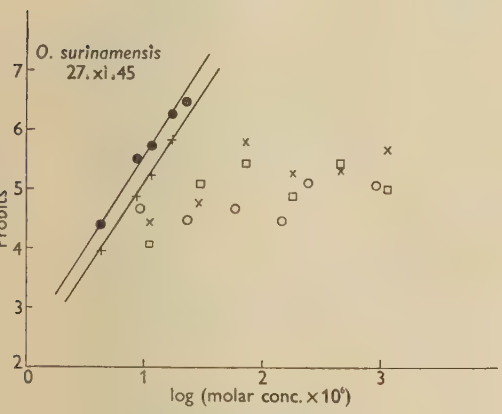

Fig. 3

	Usual medium	1 % olive oil added
$(ClC_6H_4)_2CH.CCl_3$	●	+
$(IC_6H_4)_2CH.CCl_3$	□	×
$(IC_6H_4)_2CH.CBr_3$	○	

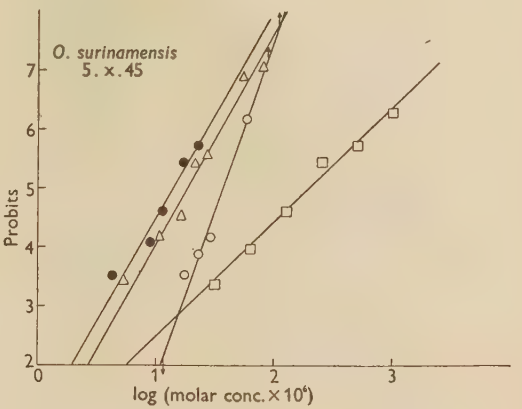

Fig. 4

● $(ClC_6H_4)_2CH.CCl_3$ ○ $(CH_3OC_6H_4)_2CH.CCl_3$
↑ 100 % mortality △ $(C_2H_5OC_6H_4)_2CH.CCl_3$
↓ 0 % mortality □ $(C_3H_7OC_6H_4)_2CH.CCl_3$

TABLE 3. *Toxicity of D.D.T. analogues*

L.D. 50's as log (molar concentrations × 10^6)

Substance	Test-subjects: *M. sanborni*	Weighted means	*O. surinamensis*	Weighted means
Various degrees of halogenation of diphenylethane				
$(C_6H_5)_2CH.CH_3$	2·720 ± 0·017 2·847 ± 0·020	2·773 ± 0·013	3·573 ± 0·026	—
$(ClC_6H_4)_2CH.CH_3$	2·296 ± 0·045 2·380 ± 0·023	2·363 ± 0·019	3·228 ± 0·033	—
$(C_6H_5)_2CH.CCl_3$	2·325 ± 0·023 2·296 ± 0·031	2·314 ± 0·018	—*	—
$(ClC_6H_4)_2CH.CCl_3$	0·951 ± 0·029 1·169 ± 0·044	1·017 ± 0·024	0·806 ± 0·055	—
$(ClC_6H_4)_2CH.CCl_3$	1·169 ± 0·044 1·221 ± 0·077	1·182 ± 0·038	0·966 ± 0·022 1·066 ± 0·029	1·003 ± 0·018
$ClC_6H_4.C(C_6H_5)H.CCl_3$	1·536 ± 0·047 1·521 ± 0·036	1·523 ± 0·029	2·434 ± 0·030 2·495 ± 0·039	2·457 ± 0·024
$(ClC_6H_4)_2CH.CHCl_2$	1·898 ± 0·059 1·549 ± 0·173	1·862 ± 0·056	1·514 ± 0·040 1·569 ± 0·045	1·538 ± 0·030
$(ClC_6H_4)_2CCl.CCl_3$	—*	—	—*	—
$(ClC_6H_4)_2C{=}CCl_2$	—*	—	—*	—
Halogen analogues				
$(FC_6H_4)_2CH.CCl_3$	1·452 ± 0·230 1·435 ± 0·060	1·436 ± 0·058	1·569 ± 0·039	—
$(ClC_6H_4)_2CH.CCl_3$	1·430 ± 0·068 1·373 ± 0·046	1·391 ± 0·038	0·940 ± 0·037 0·869 ± 0·032 0·838 ± 0·034	0·879 ± 0·020
$(BrC_6H_4)_2CH.CCl_3$	1·518 ± 0·081 1·504 ± 0·090	1·512 ± 0·060	0·828 ± 0·037 0·726 ± 0·048 0·895 ± 0·044	0·822 ± 0·024
$(IC_6H_4)_2CH.CCl_3$	—*	—	—*	—
$(FC_6H_4)_2CH.CBr_3$	2·348 ± 0·087 2·000 ± 0·033	2·044 ± 0·031	2·049 ± 0·038	—
$(ClC_6H_4)_2CH.CBr_3$	2·047 ± 0·065 2·375 ± 0·073	2·192 ± 0·049	1·746 ± 0·049	—
$(BrC_6H_4)_2CH.CBr_3$	—*	—	1·286 ± 0·121 1·746 ± 0·071	1·628 ± 0·061
$(IC_6H_4)_2CH.CBr_3$	—	—	—*	—
Alkyloxy analogues				
$(HOC_6H_4)_2CH.CCl_3$	Non-toxic	—	Non-toxic	—
$(CH_3OC_6H_4)_2CH.CCl_3$	1·632 ± 0·060	—	1·563 ± 0·021	—
$(C_2H_5OC_6H_4)_2CH.CCl_3$	0·893 ± 0·104	—	1·264 ± 0·026	—
$(C_3H_7OC_6H_4)_2CH.CCl_3$	—*	—	2·287 ± 0·047	—
$(ClC_6H_4)_2CH.CCl_3$	1·010 ± 0·050	-	1·143 ± 0·028	—

* These substances exhibit some toxicity but owing to the nature of the biological data, it is not possible to estimate the L.D. 50's against these test-subjects.

TABLE 4. *Slopes of probit lines of D.D.T. analogues*

Substance	Test-subjects: *M. sanborni* Slope	*M. sanborni* Mean slope	*O. surinamensis* Slope	*O. surinamensis* Mean slope
Various degrees of halogenation of diphenylethane				
$(ClC_6H_4)_2CH.CCl_3$	2·68 ± 0·28 2·07 ± 0·36	2·45 ± 0·22	2·52 ± 0·45	—
$(C_6H_5)_2CH.CH_3$	11·07 ± 1·71 8·29 ± 1·99	9·98 ± 1·29	4·82 ± 0·75	—
$(ClC_6H_4)_2CH.CH_3$	3·24 ± 0·63 5·89 ± 0·86	(4·17 ± 0·51)	3·68 ± 0·52	—
$(C_6H_5)_2CH.CCl_3$	7·22 ± 1·35 4·19 ± 0·60	4·69 ± 0·55	Very small	—
$(ClC_6H_4)_2CH.CCl_3$	2·07 ± 0·36 1·35 ± 0·26	1·61 ± 0·21	4·69 ± 0·48 4·40 ± 0·75	4·60 ± 0·40
$ClC_6H_4.C(C_6H_5)H.CCl_3$	2·53 ± 0·29 3·57 ± 0·53	2·77 ± 0·26	3·16 ± 0·35 2·73 ± 0·38	2·96 ± 0·26
$(ClC_6H_4)_2CH.CHCl_2$	1·57 ± 0·18 0·71 ± 0·18	(1·15 ± 0·13)	2·45 ± 0·30 2·69 ± 0·35	2·55 ± 0·23
$(ClC_6H_4)_2CCl.CCl_3$	Very small	—	Very small	—
$(ClC_6H_4)_2C{=}CCl_2$	Very small	—	Very small	—
Halogen analogues				
$(FC_6H_4)_2CH.CCl_3$	1·15 ± 0·46 2·00 ± 0·26	1·79 ± 0·23	3·25 ± 0·39	—
$(ClC_6H_4)_2CH.CCl_3$	2·72 ± 0·52 2·36 ± 0·31	2·45 ± 0·26	3·19 ± 0·41 4·90 ± 0·67 4·00 ± 0·58	3·75 ± 0·30
$(BrC_6H_4)_2CH.CCl_3$	2·24 ± 0·54 1·13 ± 0·23	1·30 ± 0·21	2·79 ± 0·29 2·48 ± 0·37 3·05 ± 0·43	2·75 ± 0·20
$(IC_6H_4)_2CH.CCl_3$	Very small	—	Very small	—
$(FC_6H_4)_2CH.CBr_3$	1·81 ± 0·45 4·06 ± 0·51	(2·79) (± 0·34)	3·06 ± 0·44	—
$(ClC_6H_4)_2CH.CBr_3$	1·29 ± 0·35 1·68 ± 0·29	1·52 ± 0·22	3·24 ± 0·55	—
$(BrC_6H_4)_2CH.CBr_3$	Very small	—	1·05 ± 0·16 1·18 ± 0·15	1·12 ± 0·11
$(IC_6H_4)_2CH.CBr_3$	—	—	Very small	—
Alkyloxy analogues				
$(CH_3OC_6H_4)_2CH.CCl_3$	—	1·74 ± 0·31	—	5·84 ± 0·63
$(C_2H_5OC_6H_4)_2CH.CCl_3$	—	1·12 ± 0·20	—	3·60 ± 0·36
$(C_3H_7OC_6H_4)_2CH.CCl_3$	—	—	—	1·95 ± 0·22
$(C_4H_9OC_6H_4)_2CH.CCl_3$	—	Non-toxic	—	Non-toxic
$(ClC_6H_4)_2CH.CCl_3$	—	1·91 ± 0·29	—	3·50 ± 0·53

Mean values are given in brackets when they are means from values which are significantly different.

TABLE 5. *Relative potencies of D.D.T. analogues at various levels of kill*

	Test-subjects							
	M. sanborni				*O. surinamensis*			
Substance	25%	50%	75%	95%	25%	50%	75%	95%
$(C_6H_5)_2CH.CH_3$	21	34	54	110	2	3	4	7
$(ClC_6H_4)_2CH.CH_3$	45	64	82	120	4	5	6	9
$(ClC_6H_4)_2CH.CCl_3$	1000	1000	1000	1000	1000	1000	1000	1000
$(C_6H_5)_2CH.CCl_3$	44	63	84	130	—	—	—	—
$(ClC_6H_4)_2CH.CCl_3$	1000	1000	1000	1000	1000	1000	1000	1000
$ClC_6H_4.C(C_6H_5)H.CCl_3$	360	510	720	1200	46	39	33	25
$(ClC_6H_4)_2CH.CHCl_2$	270	230	120	140	410	320	250	160
$(FC_6H_4)_2CH.CCl_3$	1200	990	560	620	250	230	210	180
$(ClC_6H_4)_2CH.CCl_3$	1000	1000	1000	1000	1000	1000	1000	1000
$(BrC_6H_4)_2CH.CCl_3$	790	610	440	300	1700	910	760	580
$(FC_6H_4)_2CH.CBr_3$	130	170	230	310	590	530	470	380
$(ClC_6H_4)_2CH.CBr_3$	160	120	64	38	110	97	88	75
$(BrC_6H_4)_2CH.CBr_3$	—	—	—	—	290	110	46	11
$(CH_3OC_6H_4)_2CH.CCl_3$	270	250	230	200	330	390	470	600
$(C_2H_5OC_6H_4)_2CH.CCl_3$	2200	1200	700	310	710	720	730	740
$(C_3H_7OC_6H_4)_2CH.CCl_3$	—	—	—	—	90	63	45	27
$(ClC_6H_4)_2CH.CCl_3$	1000	1000	1000	1000	1000	1000	1000	1000

TABLE 6. *Comparison of toxicities of benzene hexachloride isomers with D.D.T.*

	M. sanborni	*O. surinamensis*
	Slopes of probit lines	
D.D.T.	$1{\cdot}42 \pm 0{\cdot}27$	$2{\cdot}74 \pm 0{\cdot}63$
α-B.H.C.	—	$3{\cdot}46 \pm 0{\cdot}94$
β-B.H.C.	—	—
γ-B.H.C.	$1{\cdot}96 \pm 0{\cdot}71$	$3{\cdot}71 \pm 0{\cdot}72$
δ-B.H.C.	$1{\cdot}81 \pm 0{\cdot}33$	$2{\cdot}86 \pm 0{\cdot}52$
	50% mortality level—log (molar concentration × 10^6)	
D.D.T.	$0{\cdot}893 \pm 0{\cdot}073$	$0{\cdot}890 \pm 0{\cdot}060$
α-B.H.C.	—	$2{\cdot}258 \pm 0{\cdot}051$
β-B.H.C.	—	—
γ-B.H.C.	$1{\cdot}041 \pm 0{\cdot}072$	$1{\cdot}434 \pm 0{\cdot}044$
δ-B.H.C.	$2{\cdot}236 \pm 0{\cdot}091$	$2{\cdot}397 \pm 0{\cdot}055$
	Relative potencies	
D.D.T.	1000	1000
α-B.H.C.	—	5
β-B.H.C.	—	—
γ-B.H.C.	870	350
δ-B.H.C.	55	4

SUBSTITUTION OF CHLORINE IN VARIOUS PARTS OF THE DIPHENYLETHANE MOLECULE

Slopes of probit regression lines

M. sanborni *as test-subject*. Substitution of chlorine in both aliphatic and aromatic parts of the diphenylethane molecule progressively reduces the slope of the probit regression lines. So that, in order of slopes, the substances may be arranged:

$$(C_6H_5)_2CH.CH_3 > (ClC_6H_4)_2CH.CH_3 = (C_6H_5)_2CH.CCl_3 > ClC_6H_4.C(C_6H_5)H.CCl_3 > (ClC_6H_4)_2CH.CCl_3.$$

Substitution in the two parts of the molecule does not have equal effect on the slopes of the probit line, as is shown by the differences in slopes of the two structural isomers, the slope of the line obtained with the one substance being about twice as steep as that obtained with the other:

$$ClC_6H_4.C(C_6H_5)H.CCl_3 > (ClC_6H_4)_2CH.CHCl_2.$$

The slope of the D.D.T. line is intermediate between the two.

O. surinamensis *as test-subject*. A more complicated state of affairs arises when *O. surinamensis* is the test-subject. In this case the substitution of chlorine in the aromatic part of the diphenylethane group has a smaller effect on the slope of the probit line. Substitution in the aliphatic part of the molecule reduces toxicity below a measurable level, so that no numerical estimate of the slope of probit line can be obtained. The introduction of chlorine into the aromatic part of the molecule increases toxicity and results in the slope of the probit line becoming steeper. Although few data are available, owing to the lack of toxicity of the substance $(C_6H_5)_2CH.CCl_3$, it appears that the slope of the 'probit line' obtained with this substance is very small. Thus

$$(C_6H_5)_2CH.CCl_3 < ClC_6H_4.C(C_6H_5)H.CCl_3 < (ClC_6H_4)_2CH.CCl_3.$$

Toxicity comparisons

Because toxicity differences are so large, quantitative comparisons show little difference whether made on the basis of gram concentration or molecular concentration.

M. sanborni *as test-subject*. The toxicity of the diphenyl ethane molecule increases with progressive introduction of chlorine atoms. Thus the toxicities may be arranged in the ascending order

$$(C_6H_5)_2CH.CH_3 < (ClC_6H_4)_2CH.CH_3 = (C_6H_5)_2CH.CCl_3 < ClC_6H_4.C(C_6H_5)H.CCl_3 < (C_6H_4Cl)_2CH.CCl_3.$$

The order is liable to be modified to a certain extent, however, depending on the mortality level at which comparisons are made. Thus, at high mortality levels, $ClC_6H_4.C(C_6H_5)H.CCl_3$ appears to be more toxic than $(ClC_6H_4)_2CH.CCl_3$ (D.D.T.). The position of the substituents in the molecule also affects the potency considerably, as can be seen from the fact that the toxicity of the substance

$ClC_6H_4.C(C_6H_5)H.CCl_3$ is much higher than that of its structural isomer $(ClC_6H_4)_2CH.CHCl_2$.

O. surinamensis *as test-subject*. The toxicity of diphenyl ethane is not greatly enhanced by the introduction of chlorine into the *p*-position of the phenyl groups, whereas the introduction of chlorine to the aliphatic part of the molecule reduces toxicity to below measurable levels. However, substitution of chlorine in the *p*-position of the aromatic part of the diphenyl trichloroethane molecule increases toxicity considerably, and in order of toxicity the substances may be arranged

$$(C_6H_5)_2CH.CCl_3 < ClC_6H_4.C(C_6H_5)H.CCl_3 < (ClC_6H_4)_2CH.CCl_3,$$

as is also shown by the toxicities of the following substances which may be summarized

$$(ClC_6H_4)_2CH.CH_3 = (C_6H_5)_2CH.CH_3 > (C_6H_5)_2CH.CCl_3,$$

and the compound $(ClC_6H_4)_2CH.CCl_3$ is two to seven times as potent as $(ClC_6H_4)_2CH.CHCl_2$, but is 20–40 times as potent as $ClC_6H_4.C(C_6H_5)H.CCl_3$, while the potency of $(C_6H_5)_2.CH.CCl_3$ is too low to obtain an estimate.

Comparison of the two test-subjects

The introduction of chlorine into the diphenyl ethane molecule has a greater effect on the slope of the probit lines obtained when *M. sanborni* is test-organism than when *O. surinamensis* is used; on the other hand, when toxicity is considered the reverse effect is to be noted. It is interesting to compare the relative toxicities of the two structural isomers to the two test-organisms since the results definitely show that the relative toxicities of D.D.T. analogues are not parallel as regards different insect species. Thus the compound $ClC_6H_4.C(C_6H_5)H.CCl_3$ is approximately seven times as toxic to *M. sanborni* as its isomer $(ClC_6H_4)_2CH.CHCl_2$, but the reverse holds true against *O. surinamensis*. These differences are too great to be ascribed to chance.

At high mortality levels the substance $ClC_6H_4.C(C_6H_5)H.CCl_3$ has a potency comparable with that of D.D.T. to *M. sanborni*, but possesses only about one-fortieth of the activity of D.D.T. against *O. surinamensis*. It is possible that this substance might, in practice, effect a greater degree of control than D.D.T. in the case of certain types of insects, such as aphides.

The data obtained using the two substances

$$(ClC_6H_4)_2C{=}CCl_2 \text{ and } (ClC_6H_4)_2CCl.CCl_3$$

against both *M. sanborni* and *O. surinamensis* are unsatisfactory for probit analysis (see Figs. 4 and 5). Against both insects, however, the unsaturated compound does show considerable toxicity, but an increase in the dosage applied does not give the expected increase in mortality. The same is true of the other compound which, however, shows only slight activity.

Halogen analogues of D.D.T.

Slopes of probit regression lines

Within the range of the compounds tested the effects of substituting other halogens for chlorine in the D.D.T. molecule on the slopes of the probit line are similar, when either *M. sanborni* or *O. surinamensis* is used as a test-subject, and qualitative observations applying to one species are usually applicable to the other.

Except in the case of fluorine, which is apparently anomalous, the slope of the probit lines becomes flatter with increasing molecular weight, in the series of compounds derived by the substitution of the halogens in the *p*-positions of 1, 1, 1-trichloro-2, 2-diphenylethane. The 'probit lines' obtained with the iodo compounds tend to be horizontal. Similar results were obtained when the corresponding derivatives of diphenyl tribromoethane were tested against *O. surinamensis*, but when the test-subjects were *M. sanborni* the probit lines obtained were in general much flatter, and the one for 1, 1-*bis* (*p*-bromophenyl) 2, 2, 2-tribromoethane was horizontal. The data obtained with 1, 1-*bis* (*p*-fluorphenyl 2, 2, 2-tribromoethane against *M. sanborni* are not entirely satisfactory, but it appears that the slope of this line is steeper than that of the corresponding dichloro-tribromo compound.

It was considered possible that the flatness of the probit lines obtained with the iodo compounds was due to their relative insolubility. Olive oil at 1 % was therefore added to the spray medium in an attempt to maintain the iodo compound in solution and to facilitate penetration into the insect. The toxicity of the iodo compound was not significantly altered, although the toxicity of D.D.T. was apparently reduced by a small amount.

Toxicity comparisons

In making comparisons of toxicity of this group of substances it is particularly essential to take into account the relative slopes of the probit regression lines obtained, since in all but one of the cases under consideration the slope of the line is much less than that of D.D.T. Thus in two cases the potencies at low mortality levels are higher than those for D.D.T., but at high mortality levels considerably less.

The general trend of toxicities of these compounds to both *M. sanborni* and *O. surinamensis* is similar. The difluor-trichlor-derivative excepted, the toxicity decreases with increasing molecular weight. It is important to notice that the toxicity towards *M. sanborni* appears to fall off more rapidly with increasing molecular weight than it does towards *O. surinamensis*, and that the toxicity to *M. sanborni* of the difluor-trichlor compound relative to D.D.T. is greater than to *O. surinamensis*. The reverse holds true in the case of the dibromo-trichlor compound.

Alkyloxy analogues of D.D.T.

Slopes of probit regression lines

Similar trends are to be observed in the slopes of the probit lines obtained using both *M. sanborni* and *O. surinamensis*, although the slopes of the lines obtained with *M. sanborni* are consistently less than those obtained with the same substance against *O. surinamensis*. The slopes of the probit lines decrease with increasing molecular weight without exception.

Unfortunately, the necessary range of concentrations to give a probit regression line with the propoxy derivative was not correctly gauged against *M. sanborni*, and insufficient material was available to carry out further tests. The evidence available, however, suggests a very flat probit regression line.

Toxicity comparisons

The toxicity to both *M. sanborni* and *O. surinamensis* of poisons in the series appears to be at a maximum in the ethoxy compound, with toxicity decreasing with further increases in molecular weight: this is very similar to the observation made on the series of halogen analogues.

The compound $(HOC_6H_4)_2CH.CCl_3$, which may be considered as the first member of the homologous series, shows no measurable activity.

General trends in toxicity

Apart from certain differences in toxicity to the two test-subjects which have been discussed under the detailed examination of the various groups of compounds, the effects of the poisons tested on the two species of insects show decidedly similar trends. Therefore, in discussing them, differences in the reaction of the two test-subjects have been neglected in most instances.

General relationship between toxicities of the various substances

The general relationship between the toxicities of the various poisons and groups of poisons can best be seen from the plot of the relative potencies (at the 50% mortality level or median lethal concentration) against molecular weight (Fig. 5). The general form of the plot is similar for both species of insects.

The maximum toxicity of the compounds to both insect species appears to occur somewhere in the molecular weight range of 300–450. On either side of this range the toxicity falls off rapidly. Similar observations have been made by other workers using a series of poisons. Thus Tattersfield & Gimingham (1927) showed that the toxicity of fatty acids, their salts and esters showed an increase in toxicity with molecular weight up to a certain point in the series, after which toxicity diminished. Similar results were obtained by Dills & Menusan (1935) who also worked on the use of fatty acids and their soaps as contact insecticides. Again the toxicity of the *n*-alkyl thiocyanates increases with molecular weight to a certain point in the series

and then decreases with further increases in molecular weight (Bousquet, Salzberg & Dietz, 1935). Comparisons of a very precise nature are not possible in our case, owing to the wide differences in slope of the probit lines with the result that the

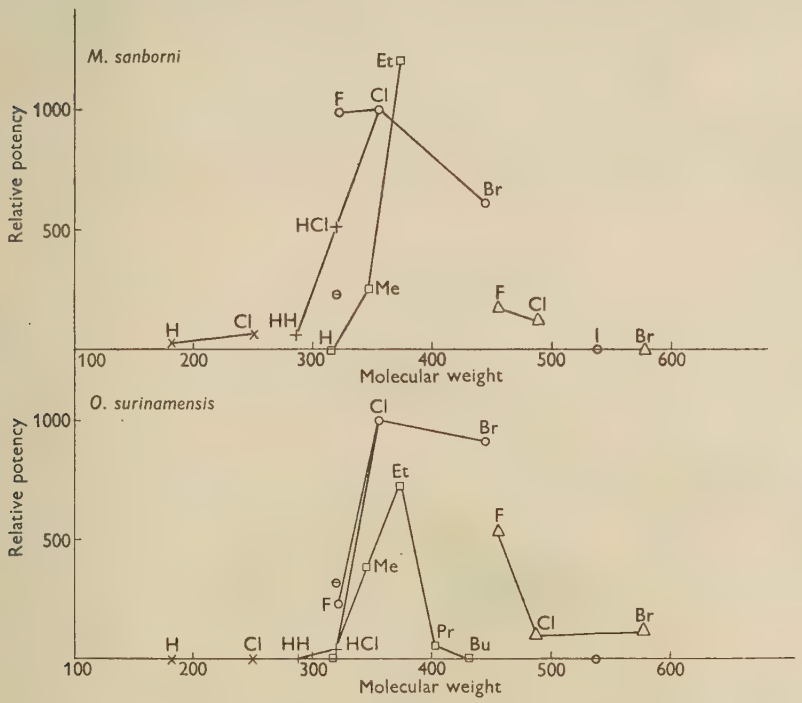

Fig. 5. Relationship between molecular weight and relative potencies of D.D.T. analogues at 50 % mortality levels (L.D. 50).

$(XC_6H_4)_2CH.CCl_3$: X = F	○	F	$ClC_6H_4.C(C_6H_5)H.CCl_3$	H	+ HH
Cl	○	Cl	$ClC_6H_4.C(C_6H_5)H.CCl_3$		+ HCl
Br	○	Br	$(ClC_6H_4)_2CH.CHCl_2$		⊖
I	○	I	$(C_6H_5)_2CH.CH_3$		× H
OH	□	H	$(ClC_6H_4)_2CH.CH_3$		× Cl
OCH_3	□	Me	$(XC_6H_4)_2CH.CBr_3$: X = F		△ F
OC_2H_5	□	Et		Cl	△ Cl
OC_3H_7	□	Pr		Br	△ Br
OC_4H_9	□	Bu		I	△ I

relative potencies at various mortality levels are widely different. Plotting relative potencies at L.D. 50 against molecular volumes (parachor)* does not profoundly alter the relationships.

* The parachor is a measure of molecular volume and for most substances is equal to 0·77 times the molecular volume at the critical temperature. In our case the values of the parachor were calculated from the constants given by Mumford & Phillips (1929).

General relationships between slopes of probit lines of the various substances

Plotting the relative slopes of probit lines against molecular weight of poisons reveals a general tendency for flatter slopes with increasing molecular weight. If, instead of molecular weight, the parachor is used to set out the data (Fig. 6) the

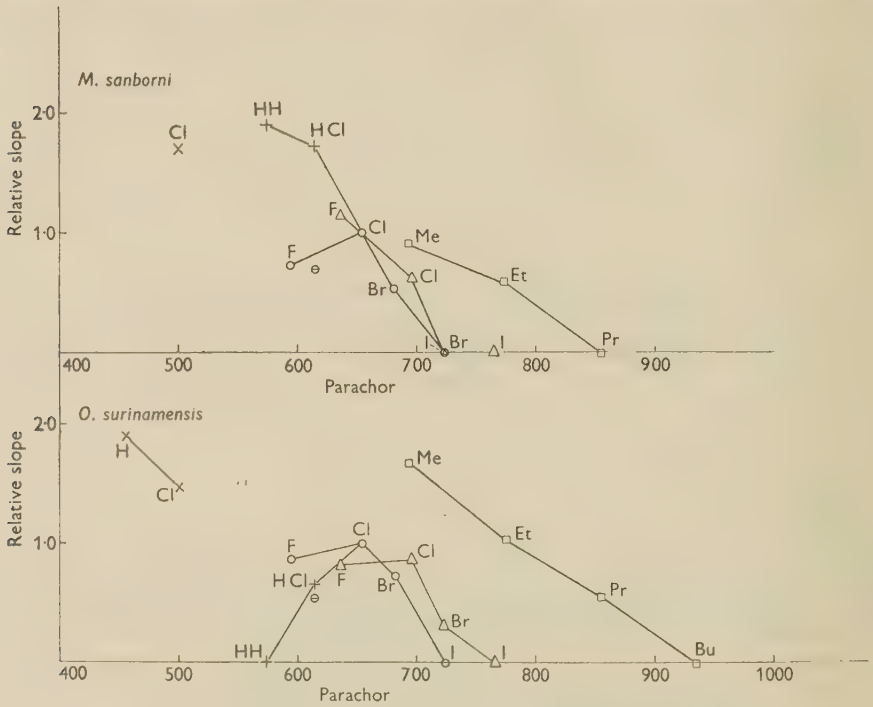

Fig. 6. Relationship between parachor and slopes of probit lines of D.D.T. analogues.

$(XC_6H_4)_2CH.CCl_3$: X=F	○ F	$ClC_6H_4.C(C_6H_5)H.CCl_3$	+ HCl
Cl	○ Cl	$(ClC_6H_4)_2CH.CH_3Cl_2$	⊖
Br	○ Br	$(C_6H_5)_2CH.CH_3$	× H
I	○ I	$(ClC_6H_4)_2CH.CH_3$	× Cl
OH	□ H	$(XC_6H_4)_2CH.CBr_3$: X=F	△ F
OCH_3	□ Me	Cl	△ Cl
OC_2H_5	□ Et	Br	△ Br
OC_3H_7	□ Pr	I	△ I
OC_4H_9	□ Bu		
H	+ HH		

general distribution is maintained, but the lines joining members of the same series of compounds become very nearly parallel.

The values for the two groups of compounds derived from diphenyl trichlorethane and diphenyl tribromethane are almost superposed. It seems, therefore, that the

slope of the probit line is more closely related to the molecular volume of the poison than to the molecular weight.

The only apparently systematic exception to the general trend of increasing slope with decreasing molecular weight is that exhibited by the compounds

$$(C_6H_5)_2CH.CCl_3, \quad ClC_6H_4.C(C_6H_5)H.CCl_3, \quad (ClC_6H_4)_2CH.CCl_3,$$

which when tested against *O. surinamensis* showed the reverse trend.

There is a general trend for the slopes obtained against *O. surinamensis* to be steeper than those obtained against *M. sanborni*. The slopes obtained with two substances $(C_6H_5)_2CH.CH_3$ and $(C_6H_5)_2CH.CCl_3$ definitely show the reverse effect.

The toxicity of the isomers of benzene hexachloride (B.H.C.)

(1, 2, 3, 4, 5, 6-*hexachlorocyclohexane*)

The four isomers of B.H.C. were assayed for their toxicity by contact against the same two insect species, *M. sanborni* and *O. surinamensis*, and in the same medium as the D.D.T. analogues. The results of these toxicity trials are summarized in Table 6.

The solubility of the β-isomer in benzene is so small that it has not been possible to obtain a spray preparation, in the medium used, sufficiently toxic to obtain a probit regression line with either species. Similarly, the α-isomer was not sufficiently soluble to obtain a line when tested against *M. sanborni*.

The slopes of the probit lines obtained by using the different isomers against the same test-subject were not significantly different, and the lines appeared to be parallel to those obtained with D.D.T.

Since all the lines are parallel it is possible to make comparisons at one mortality level only. Against *M. sanborni* the γ-compound is far more toxic than any of the other isomers, of which the δ-derivative appears to be most potent, the others being too insoluble to obtain a sufficiently concentrated solution to determine their toxicity. In the medium used, the toxicity of the γ-isomer appears to be about nine-tenths of that of D.D.T. to *M. sanborni*. Against *O. surinamensis* the γ-isomer is again far more toxic than the other isomers, but is only about one-third as toxic as D.D.T.

It should be noted that the relative potencies of the isomers of B.H.C. against the two insect species are not the same, thus, the relative potencies of the α- γ- and δ-isomers against *M. sanborni* are as 0:16:1, and against *O. surinamensis* as 1·2:88:1 respectively.

Significance of results

Slopes. The slope of a probit regression line depends partly on the scatter of the resistance of the individuals within the population and partly on the accuracy of the administration of a dose to individual insects, and therefore if either of these factors changes an alteration in slope of the probit line will result. Here, however, the

differences in slope of the probit lines described cannot be explained entirely on the basis of variations in the accuracy of application of the poisons (applied by the same method) and must therefore be ascribed in part to biological variation. The term 'biological variation' is used here in its widest possible sense and is taken to include not only general variations in biological material, but also specific variations in the reaction of similar biological material to treatment with different poisons.

It has been observed that the slopes of the probit lines obtained when *M. sanborni* is treated with D.D.T. are considerably and consistently flatter than when *O. surinamensis* is the test-subject. It is obvious in this case that the result is due to different biological material, and that the variance of the tolerance to D.D.T. in the individuals of the two species is different.

The relative slopes of the probit lines obtained by using the three substances $(ClC_6H_4)_2CH.CCl_3$, $ClC_6H_4.C(C_6H_5)H.CCl_3$ and $(C_6H_5)_2CH.CCl_3$ against *M. sanborni* are as 1:1·72:1·91 and against *O. surinamensis* are as 1:0·64:0·00.

If the relative slopes of the probit lines of these substances were similar with respect to both test-subjects it would be possible to draw the conclusion that the toxic materials incorporated in the spray fluid influenced its physical properties in such a way that their differences could be ascribed to changes in the accuracy of dosage delivery. Since this is not the case it must be concluded that the relative tolerances of the individuals of a species are different to different poisons. This conclusion is further supported by comparing the slopes of the probit lines obtained by testing the substances $(ClC_6H_4)_2CH.CCl_3$, $(CH_3OC_6H_4)_2CH.CCl_3$ and $(C_2H_5OC_6H_4)_2CH.CCl_3$ against *M. sanborni* when the relative slopes are as 1:0·91:0·59 and against *O. surinamensis* when the relative slopes are as 1:1·67:1·03.

Discussion of the toxicity of D.D.T. and its analogues

Neither of the two theories which have been advanced to explain the toxicity of the D.D.T. molecule is able to account for the toxicity of all the related compounds.

Lauger, Martin & Muller (1944) have suggested that the toxophoric group in the D.D.T. molecule is the *bis*-(*p*-chlorophenyl) system and postulate that the function of the CCl_3 is to give the molecule sufficient lipoid solubility to reach its site of action. Martin & Wain (1944*b*), however, consider this theory unsatisfactory since it fails to account for the lack of toxicity of the compound $(ClC_6H_4)_2CCl.CCl_3$ which possesses both the chlorophenyl system and the CCl_3 group. Busvine (1946) again regards the theory as unconvincing since it fails to account for the low toxicity of the substances $(ClC_6H_4)_2C{=}CCl_2$ and $(ClC_6H_4)_2CH.CH_3$ which are both more oil-soluble than D.D.T. and possess the chlorophenyl system which Lauger postulates as the toxophoric grouping.

Martin & Wain (1944*a*) suggest that the toxophoric group of D.D.T. is the $CH.CCl_3$ group, having the ability to liberate hydrochloric acid on decomposition

$$\rangle CH.CCl_3 \rightarrow \rangle C{=}CCl_2 + HCl.$$

They conclude that D.D.T. possesses its insecticidal properties because it satisfies the three following conditions: (1) ability to penetrate and concentrate at site of action; (2) adequate stability to reach site of action; (3) ability to release hydrochloric acid when adsorbed at the sensitive interface within the organism.

Busvine (1946), however, considers that this theory is unsatisfactory owing to the lack of correlation between the hydrolysis constants and toxicities of the various analogues, and remarks that in general the most inactive analogues are more oil-soluble than D.D.T.

In addition, the theory takes no account of the fact that $(ClC_6H_4)_2CH.CH_3$ has a measure of toxicity which on the basis of this theory should be non-toxic, since the possibility of liberation of HCl is excluded in this case.

The two theories are similar in that they both attempt to relate the toxicity of the compounds with one part of the molecule, and while they recognize the importance of lipoid solubility, no attempt is made to relate any of the physical properties of the various substances with their toxicities. If, however, the view is taken, as suggested by Busvine (1946), that the toxicity is due to the molecule as a whole, the most toxic derivatives of diphenyl ethane being most similar in size and shape to D.D.T., then it must be concluded that the main action of the insecticide arises from physico-chemical causes rather than from a pure chemical reaction. It is not possible, however, in the present state of our knowledge to arrive at any definite conclusion on this point.

The existence of a direct relationship between certain physical properties and insecticidal activity has been suggested by several workers. Thus toxicity towards insects has been related with boiling-point (Moore, 1917*a*, *b*), vapour pressures (Tattersfield & Roberts, 1920), to the partition coefficient between oil and water (Tattersfield & Gimingham, 1927), and to adsorption and surface activity (O'Kane, Westgate, Glover & Lowry, 1930). Ferguson (1939) correlated these results with those obtained in other fields and expanded the ideas involved and made them more precise in his concept of chemical potentials. The criteria capable of determining whether the toxicity of a group of compounds is of a physical or chemical nature are, however, not available for this series of substances. The relationship between the potency and degree of substitution of similar chlorine derivatives of diphenyl ethane (see Fig. 7) are, however, suggestive of the relationships established between the toxicity of members of homologous series.

In view of the similarity between the action of D.D.T. and γ-B.H.C., as shown by the experiments on the effect of poisons on insect respiration (Lord, 1949), any theory as to the toxicity of D.D.T. and its analogues should also include the toxicity of B.H.C. On the theory of Martin & Wain (1944*a*, *b*), B.H.C. is expected to be toxic by virtue of its loss of hydrochloric acid when treated with alkalis. But this theory does not give a ready explanation of the difference in toxicity of the various isomers of B.H.C.

Slade (1945) has suggested that the toxicity of γ-B.H.C. is due to its similarity

to *i*-inositol in size and molecular configuration and certain physical properties and its consequent ability to interfere with inositol metabolism.

Kirkwood & Phillips (1946), working with *Saccharomyces cerevisiae*, and Buston, Jacobs & Goldstein (1946), working with *Nematospora gossypii*, have demonstrated that the inhibition of growth of their test organisms by γ-B.H.C. is reversed by the addition of excess of *i*-inositol. The other isomers are very much less active. If γ-B.H.C. is toxic to insects because it interferes with the metabolism of *i*-inositol then this would appear to involve a reversible action. It is not unreasonable to suppose that D.D.T. acts by a similar interference mechanism, as suggested by Slade.

A mechanism of this type would require the toxic molecule to possess certain physico-chemical properties rather than any specific groups, and toxicity would thus depend upon the molecule as a whole rather than upon any particular part of the molecule.

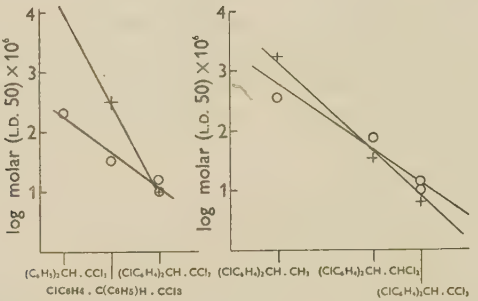

Fig. 7. Relationship between log (molar (L.D. 50) × 10^6) and degree of substitution.

Test subjects: ○ *M. sanborni*, + *O. surinamensis*.

In Fig. 7 the L.D. 50's (log molar concentrations) are plotted against degree of substitution of chlorine atoms and an approximately linear relationship is shown. If the toxicity is indeed due to some physical mechanism, then this relationship between toxicity and the degree of substitution of intermediate members of the series of substances between *bis*-(*p*-chlorphenyl)ethane and D.D.T. and diphenyl trichloroethane and D.D.T. is not unreasonable. Also the not widely different median lethal concentrations of the *p*-substituted analogues of diphenyltrichloroethane fall into line. Moreover, the sorption of D.D.T. and its analogues by chitin (Lord, 1948*a*) show that these behave similarly towards a sorbing substance, which is at least not at variance with the postulate that they act by a similar physical mechanism based on molecular configuration and certain physical properties.

In addition, freezing-point curves of mixtures of D.D.T. with a number of amino compounds have not yielded any evidence of true compound formation (Martin & Wain, 1944*b*), although data accumulated in a study of the order of reaction between D.D.T. and monomethylamine indicates the formation of a complex

between the two (Lord, in preparation). The case, however, may be one of molecular association resulting from a specific molecular configuration similar to that proposed by Slade for B.H.C.

It would appear reasonable, therefore, to suppose that the biological activity of D.D.T. and B.H.C. as contact insecticides results from a reversible physico-chemical action rather than any irreversible chemical reaction, and that the intensity of biological activity is dependent on the physico-chemical properties of the molecule, although the compounds may be dependent for their toxicity on a specific molecular configuration.*

I wish to acknowledge the interest and advice of Dr Tattersfield throughout the work described here. My thanks are also due to the Ministry of Supply who kindly presented the substances listed in Table 2 and to Imperial Chemical Industries, Ltd., who generously supplied the isomers of benzene hexachloride.

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* Since the above statement was written the author's attention has been drawn to a paper by Welsh & Gorden (1947) in which they suggest, as a result of work on the action of a number of diverse chemicals on arthropod nerves, that the *primary* action of D.D.T., B.H.C. and a number of other insecticides is physical and not chemical.

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RELATION BETWEEN BIOASSAY SYSTEMS AND THE VALUES FOUND FOR TOXICITY OF D.D.T.

By A. STRINGER
Long Ashton Research Station, University of Bristol

The toxicities of various D.D.T. suspensions, tested by McIntosh (1947), are shown to be equivalent when compared on a weight basis. The relation $w = K\lambda^f$, connecting weight retained (w) and concentration (λ), is in better agreement with the data than McIntosh's $w = F\lambda$.

When the dosage is expressed as a concentration the slope of the regression line is dependent upon the method of testing and is a measure of the sensitivity of the system used. Experiments with *Calandra granaria* L., using three different test systems, show this variation of sensitivity and also the independence of the rate of diffusion of the insecticide through the insect cuticle.

In a recent paper McIntosh (1947) has discussed the relation between toxicity and particle size and shape in D.D.T. suspensions. Five types of crystal suspensions, differing in crystal size and shape, and colloidal D.D.T. were tested against *Tribolium castaneum* Hb., using a dipping technique. He concluded that there are indications of the toxicity of the crystals *in situ* being independent of size, and that the most important factor was the amount of D.D.T. retained by the insects. Two extreme types, colloidal and needle aggregates, were of almost equal toxicity when compared on the weights of D.D.T. picked up. Further, he questioned generalizations leading from a comparison of these two types because the slope of the regression line for the colloid was significantly different from the slopes for crystalline suspensions.

Examination of the colloidal (type I) and the needle aggregate (type IV) suspensions in test (M) points to the conclusion that the regression lines are equivalent when compared on a weight basis. McIntosh gives the following equations for the regression of probit kill on log (concentration, w/v) as

$$\text{Type I} \quad y = 1{\cdot}94x - 0{\cdot}43, \qquad \text{Type IV} \quad y = 3{\cdot}24x - 0{\cdot}66,$$

and $m_1 = 2{\cdot}80 \pm 0{\cdot}045$, $m_4 = 1{\cdot}75 \pm 0{\cdot}029$, $M_{41} = 1{\cdot}05 \pm 0{\cdot}053$ (statistical notation as recommended by Finney (1947)). Thus the assay gives the L.D. 50 of needle aggregates as eleven times as toxic as the L.D. 50 of colloidal D.D.T. when the dose is measured in terms of concentration (w/v). The weight of D.D.T. picked up (w) was determined for various concentrations (λ), and using the relation $w = F\lambda$ McIntosh found $F_1 = 0{\cdot}22$ and $F_4 = 2{\cdot}62$. The regression of probit kill on log (weight), $z = \log(w)$, gives:

$$\text{Type I} \quad y = 1{\cdot}94z + 0{\cdot}84, \qquad \text{Type IV} \quad y = 3{\cdot}24z - 2{\cdot}02,$$

and $m_1 = 2{\cdot}14 \pm 0{\cdot}045$, $m_4 = 2{\cdot}17 \pm 0{\cdot}029$, $M_{41} = -0{\cdot}03$. Thus a transformation based on a linear relationship between w and λ gives lines of different slope and equitoxic

median doses. McIntosh states that the two types can now be considered as having identical toxicities, but in view of the significant slope difference this cannot be so; for example, the ratio of toxicities (Type IV/Type I) at L.D. 50 is 1·07 and at L.D. 90 it is 1·74.

It is obvious that a change of scale based on $w = F\lambda$ will only alter the position of the regression lines and that a linear logarithmic relationship between w and λ, of the form $w = K\lambda^f$, is required to obtain a change of slope. From McIntosh's data the following equations were obtained graphically:

$$\text{Type I} \quad \log(w) = 0{\cdot}71 \log(\lambda) + 0{\cdot}10 \text{ or } w = 1{\cdot}26\lambda^{0{\cdot}71},$$

$$\text{Type IV} \quad \log(w) = 1{\cdot}20 \log(\lambda) + 0{\cdot}10 \text{ or } w = 1{\cdot}26\lambda^{1{\cdot}20}.$$

Using the new relationship $z = \log(w) = f \log(\lambda) + k$, the original regression lines when corrected for retention give the following equations:

$$\text{Type I} \; . \; y = 2{\cdot}73z - 0{\cdot}70, \quad \text{Type IV} \quad y = 2{\cdot}70z - 0{\cdot}93,$$

and $m_1 = 2{\cdot}09 \pm 0{\cdot}032$, $m_4 = 2{\cdot}20 \pm 0{\cdot}035$, $M_{41} = -0{\cdot}11$.

The slopes and the positions of the median lethal dosages are not significantly different.

From the probit/log (concentration) regression line for test (M) the following relationship holds for equal kills:

$$1{\cdot}94x_1 - 0{\cdot}43 = 3{\cdot}24x_4 - 0{\cdot}66, \quad x_1 = 1{\cdot}67x_4 - 0{\cdot}23.$$

Now $b_4/b_1 = 1{\cdot}67$ and $f_4/f_1 = 1{\cdot}69$, therefore it seems obvious that the retention coefficients, f, are related to the regression coefficients in the same way for both types of suspension. The value of b for test (L) is unexpectedly high, 5·13, and gives a value of 2·85 for the ratio b_4/b_1. The mean value of the regression coefficient for all the tests on type IV crystals is 3·63 so that $\bar{b}_4/\bar{b}_1 = 1{\cdot}81$, and if test ($L$) is omitted $\bar{b}_4 = 3{\cdot}25$ and $\bar{b}_4/\bar{b}_1 = 1{\cdot}63$ which is now approximately equal to f_4/f_1.

The relative consistency of the colloid probit/log (conc.) equations implies that the retention of D.D.T. is fairly constant from test to test and that the variations of needle aggregate suspensions are due mainly to variations of the amount of D.D.T. picked up in the different tests. McIntosh noted day-to-day variation in micro-crystal size. Less variation of particle size would be expected in the colloid than in the suspensions and it is reasonable to assume that the amount of D.D.T. retained by the insects after dipping would vary less with the colloidal than with the needle aggregate suspension.

If, in the tests carried out by McIntosh, one assumes that the level of susceptibility of the insects remained moderately constant, and the tests with the colloid suspensions indicate that this was so, then the pooled data would give average slopes and median lethal doses as in Table 1, from which it appears that the colloid and the needle aggregates are equitoxic when the kill is measured against the amount of D.D.T. acting on the insects.

The dipping technique may be regarded as a single bioassay system if one is

TABLE 1. *Dosage-mortality data for types I and IV suspensions* (from McIntosh, 1947)

Test		Equation	b	m		$(m_1 - m_4)$
		Type I. Colloid				
J	Original equations	$y = 2{\cdot}08x - 0{\cdot}79$	$2{\cdot}08 \pm 0{\cdot}35$	$2{\cdot}78 \pm 0{\cdot}046$	Average slope = 2·00	1·18
K		$y = 2{\cdot}19x - 1{\cdot}43$	$2{\cdot}19 \pm 0{\cdot}34$	$2{\cdot}94 \pm 0{\cdot}052$	—	(1·12)
L		$y = 1{\cdot}80x - 0{\cdot}31$	$1{\cdot}80 \pm 0{\cdot}21$	$2{\cdot}95 \pm 0{\cdot}032$	Average m = 2·87	—
M		$y = 1{\cdot}94x - 0{\cdot}43$	$1{\cdot}94 \pm 0{\cdot}34$	$2{\cdot}80 \pm 0{\cdot}045$	—	—
J	Using $w = F\lambda$	$y = 2{\cdot}08z + 0{\cdot}58$	—	$2{\cdot}12 \pm 0{\cdot}046$	Average slope = 2·00	−0·10
K		$y = 2{\cdot}19z + 0{\cdot}02$	—	$2{\cdot}28 \pm 0{\cdot}052$	—	(−0·11)
L		$y = 1{\cdot}80z + 0{\cdot}88$	—	$2{\cdot}29 \pm 0{\cdot}032$	Average m = 2·21	—
M		$y = 1{\cdot}94z + 0{\cdot}84$	—	$2{\cdot}14 \pm 0{\cdot}045$	—	—
J	Using $w = F\lambda'$	$y = 2{\cdot}93z - 1{\cdot}08$	$2{\cdot}93 \pm 0{\cdot}49$	$2{\cdot}07 \pm 0{\cdot}033$	Average slope = 2·82	0·02
K		$y = 3{\cdot}08z - 1{\cdot}74$	$3{\cdot}08 \pm 0{\cdot}48$	$2{\cdot}19 \pm 0{\cdot}037$	—	(−0·01)
L		$y = 2{\cdot}53z - 0{\cdot}56$	$2{\cdot}53 \pm 0{\cdot}30$	$2{\cdot}20 \pm 0{\cdot}023$	Average m = 2·14	—
M		$y = 2{\cdot}73z - 0{\cdot}70$	$2{\cdot}73 \pm 0{\cdot}48$	$2{\cdot}09 \pm 0{\cdot}032$	—	—
		Type IV. Needle aggregates				$(b_4 - b_1)$
A	Original equations	$y = 2{\cdot}70x + 0{\cdot}64$	$2{\cdot}70 \pm 0{\cdot}11$	$1{\cdot}61 \pm 0{\cdot}035$	Average slope = 3·63	1·63
B		$y = 4{\cdot}20x - 2{\cdot}46$	$4{\cdot}20 \pm 0{\cdot}42$	$1{\cdot}78 \pm 0{\cdot}023$	(3·25)	(1·25)
C		$y = 2{\cdot}87x + 0{\cdot}19$	$2{\cdot}87 \pm 0{\cdot}36$	$1{\cdot}68 \pm 0{\cdot}031$	Average m = 1·69	—
L		$y = 5{\cdot}13x - 3{\cdot}30$	$5{\cdot}13 \pm 0{\cdot}32$	$1{\cdot}62 \pm 0{\cdot}012$	(1·75)	—
M		$y = 3{\cdot}24x - 0{\cdot}66$	$3{\cdot}24 \pm 0{\cdot}43$	$1{\cdot}75 \pm 0{\cdot}029$	—	—
A	Using $w = F\lambda$	$y = 2{\cdot}70z - 0{\cdot}49$	—	$2{\cdot}03 \pm 0{\cdot}035$	Average slope = 3·63	1·63
B		$y = 4{\cdot}20z - 4{\cdot}22$	—	$2{\cdot}20 \pm 0{\cdot}023$	(3·25)	(1·25)
C		$y = 2{\cdot}87z - 1{\cdot}02$	—	$2{\cdot}10 \pm 0{\cdot}031$	Average m = 2·11	—
L		$y = 5{\cdot}13z - 5{\cdot}45$	—	$2{\cdot}04 \pm 0{\cdot}012$	(2·10)	—
M		$y = 3{\cdot}24z - 2{\cdot}02$	—	$2{\cdot}17 \pm 0{\cdot}029$	—	—
A	Using $w = K\lambda'$	$y = 2{\cdot}25z + 0{\cdot}41$	$2{\cdot}25 \pm 0{\cdot}09$	$2{\cdot}04 \pm 0{\cdot}042$	Average slope = 3·02	0·20
B		$y = 3{\cdot}50z - 2{\cdot}81$	$3{\cdot}50 \pm 0{\cdot}35$	$2{\cdot}23 \pm 0{\cdot}028$	(2·71)	(−0·11)
C		$y = 2{\cdot}39z - 0{\cdot}05$	$2{\cdot}39 \pm 0{\cdot}30$	$2{\cdot}11 \pm 0{\cdot}037$	Average m = 2·12	—
L		$y = 4{\cdot}27z - 3{\cdot}73$	$4{\cdot}27 \pm 0{\cdot}27$	$2{\cdot}04 \pm 0{\cdot}014$	(2·15)	—
M		$y = 2{\cdot}70z - 0{\cdot}93$	$2{\cdot}70 \pm 0{\cdot}34$	$2{\cdot}20 \pm 0{\cdot}035$	—	

comparing the toxicities of the different types of suspensions. But if one is estimating the toxicity of D.D.T. with respect to particle size and shape then the dipping technique must be regarded as several systems when the dosage is measured in terms of concentration, and as a single system when the dosage is expressed as the weight of D.D.T. retained by the insect. Generally, in any particular bioassay system, the amount of insecticide used by the insect is unknown and what is known is the concentration per unit volume or area of the substrate in contact with the insect. There is a tacit assumption of proportionality between the concentration and the quantity utilized. Numerous experiments with many kinds of insecticide have demonstrated an approximate normal distribution of tolerances when the doses are measured on a logarithmic scale. If a normal distribution of tolerances also holds for the log weight of insecticide at the site of action, the probit-dosage equation is

$$y = \beta \log (w) + \alpha,$$

and for a number of assay systems, k say, the equations are

$$y = b_k \log (\lambda) + a_k.$$

From these equations, it follows that $\log (w) = b_k/\beta \log (\lambda)$, and putting $f_k = b_k/\beta$, $\log (w) = f_k \log (\lambda)$. Thus f can be regarded as an 'availability' coefficient and in the comparison of any two systems, i and j, the ratio f_j/f_i, which is equal to b_j/b_i, is a measure of the relative availability of the insecticide in the two systems.

The author has examined the contact action of D.D.T. against *Calandra granaria* L. using the following methods:

(*a*) Colloidal suspensions were prepared by injecting D.D.T. alcohol solutions into water and allowing them to stand until the initial 'hydrosol' broke down to give a finely dispersed colloid. Batches of fifty *C. granaria* were placed in glass tubes, closed at both ends by muslin covers, and immersed for 1 min. in suspensions of various concentrations (ranging from 0·001 to 1·00 mg./ml.). The immersion period appeared to be unimportant; times of 1, 5, 30, 60 min. gave equal kills in suspensions containing 1 mg./ml.

The regression plane for the colloidal suspension, $x = \log$ (conc. in $10x$ mg./ml.) and $t = \log$ (time in hr.), being

$$y = 0{\cdot}841x + 5{\cdot}214t - 7{\cdot}014, \quad \text{and} \quad b_x = 0{\cdot}841 \pm 0{\cdot}058, \quad b_t = 5{\cdot}214 \pm 0{\cdot}324.$$

(*b*) Batches of fifty *C. granaria* were enclosed by means of glass rings 6 cm. in diameter on 7 cm. no. 1 Whatman filter-papers impregnated with D.D.T. deposited from acetone solutions. Dosages ranged from $3{\cdot}15 \times 10^{-6}$ to $3{\cdot}15 \times 10^{-2}$ mg./cm.^{2}, and the resulting equations were

$$y = 0{\cdot}8x - 3{\cdot}6, \quad y = 5{\cdot}55t - 6{\cdot}14, \quad b_x = 0{\cdot}8 \pm 0{\cdot}3, \quad b_t = 5{\cdot}55 \pm 0{\cdot}37.$$

(*c*) Batches of fifty insects were enclosed by glass rings on no. 1 Whatman filter-papers treated with solutions of D.D.T. in P31 petroleum oil. Each dosage

per filter-paper consisted of 0·12 ml. of oil solution, concentrations of D.D.T. varying from 0·15 to 0·5% (w/v), and the regression equations were

$$y = 2{\cdot}37x + 1{\cdot}56, \quad b = \log(100x\% \text{ conc.}), \quad y = 5{\cdot}46t - 5{\cdot}11,$$

$$b_x = 2{\cdot}37 \pm 0{\cdot}35, \quad b_t = 5{\cdot}46 \pm 0{\cdot}52.$$

Examination of the probit/log (λ) regression coefficients show that the availability of D.D.T. is of the same order for system (*a*), immersion in colloidal suspensions, and system (*b*), contact with crystalline deposits, whereas in system (*c*), where the insect is in contact with an oil-film, the availability is increased by approximately three times. The increased availability in (*c*) is attributed to the presence of the oil.

For the three systems the probit/log (time) regression coefficients were $5{\cdot}21 \pm 0{\cdot}32$ for colloidal D.D.T., $5{\cdot}55 \pm 0{\cdot}37$ for crystalline D.D.T., and $5{\cdot}46 \pm 0{\cdot}52$ for D.D.T. in P31 oil. The time regression coefficients are not significantly different, and this implies that the rate of diffusion through the cuticle to the site of action is the same for the three systems. The P31 oil film exerts little or no toxic action and Hewlett (1947) suggests that P31 oil, in virtue of its large molecules, does not pass through the cuticle. Hurst (1943) has stated that an oil-carrier has a marked influence on the degree of induced penetration, and that this influence decreases with an increase of oil viscosity. The similarity of the time slopes suggests that in the case of non-penetrating oils the cuticle has little or no increased permeability with respect to the insecticide. The lipophilic properties of P31 oil enable an oil film to be formed on the insect cuticle so that the insecticide can diffuse from the substrate oil film to the insect oil film, and the rate of diffusion in the oil film will obviously decrease with viscosity increase. The slower diffusion in the more viscous oil will result in a difference of position of the regression lines but not of a slope. Thus, with non-penetrating oils, the carrier effect is a reflexion of physical differences in the system acting externally to the insect.

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THE TOXICITY OF VAPOURS TO THE GRAIN WEEVIL

By J. FERGUSON and H. PIRIE
Imperial Chemical Industries, Ltd., Alkali and General Chemicals Division

(With 3 Text-figures)

The values for concentrations in air of the vapours of approximately one hundred different chemical substances required to produce 50% mortality in samples of the grain weevil *Calandra granaria*, have been determined. Relationships between chemical constitution of these substances and their toxicity to the grain weevil are discussed, using as a measure of toxicity the relative saturation of the vapour concentration producing 50% kill. The relative saturation is equal to the thermodynamic activity of the vapour at the concentration in question.

It is found that the substances used can be divided into two broad classes: (*a*) one class in which the toxic thermodynamic activities lie between 0·1 and 1·0 and (*b*) another in which the activities are much lower—down to 0·0004. The first class exhibits certain linear relationships between the logarithms of toxic concentration and saturated vapour pressure, which are not shown by the second class. The toxicity of the first class is ascribed to a physical mechanism, and is believed to be such that phase equilibrium relations play a predominant part in determining the value of the toxic concentration measured externally to the insect (Ferguson, 1939).

The toxicities of members of the first class, as measured by their toxic thermodynamic activities, show the following relations with chemical constitution:

(1) Toxicity in homologous series decreases steadily from the first member as the chain length increases, finally disappearing.

(2) Halogen substitution in hydrocarbon skeletons has only a small effect on the 'physical' toxicity, increasing it slightly. There is practically no difference in the effect of different halogens.

(3) Hydroxyl substitution markedly decreases 'physical' toxicity.

(4) The substitution of —CO or —CHO groups markedly increases 'physical' toxicity.

The aliphatic amines and the alkyl formates examined do not appear to act by a physical mechanism, nor do many of the alkyl bromides and iodides.

Introduction

The following paper gives the results of an investigation of the toxicities of the vapours of a number of pure substances when applied as fumigants to the grain weevil *Calandra granaria*. It is presented as a contribution to the study of the influence of chemical constitution on the toxic action of chemical compounds.

A number of such studies using insects as the biological material have been undertaken in the past. Among early investigations there may be mentioned the work of Holt (1916), who studied the toxic action of the vapours of volatile organic compounds on the cockroach. He noted that the toxicity of volatile organic compounds (measured by the reciprocals of times of exposure necessary to cause death at the same vapour concentration), appeared in general to increase with the boiling-points of the compounds, though compounds boiling above a certain critical temperature were non-toxic or feebly toxic.

Moore (1917) working on the house-fly obtained similar results. Moore, like most investigators in this field, compared the toxicities of his compounds by comparing the molar concentrations in the vapour phase which produced a definite percentage kill in a standard time. He concluded that physical characters such as boiling-point and vapour pressure have more influence on the toxicity of insect fumigants than has chemical constitution. He believed that the toxicity of a volatile organic compound is closely correlated with its volatility, decreasing volatility being accompanied by increased toxicity. He suggested that the structure of the insect's respiratory system, consisting as it does of fine capillary spiracles, might account for the 'remarkable influence of volatility' on toxicity to insects. 'The vapour present in the air is taken into the tracheae of the insects and is condensed upon reaching the finer divisions. If the compound is very volatile it will evaporate and readily pass out of the insect, while if very slightly so, it will remain in the insect and will penetrate the tissues and produce the poisonous reactions which lead to the insect's death.' In the case of higher animals, the poisonous vapour absorbed through the lungs is rapidly carried by the blood stream to all parts of the body. Hence, according to Moore, chemical constitution and reactivity will determine toxic action in such cases, and physical characters such as volatility will not be the principal determining factors.

Tattersfield & Roberts (1920) determined the lethal concentrations of a number of organic vapours towards the wireworm, *Agriotes*. They too observed a rough correlation between boiling-point and toxic concentration. Since, both in their own and Moore's experiments, the value measured was the capacity of an insect to recover from the effects of exposure during a definite period of time, they suggested that the correlation might result from the slower diffusion rates of high boiling and therefore molecularly heavy vapours out of the spiracles after exposure, a process which thus increased the effective time of exposure. They pointed out, however, that the boiling-point rule of Holt and Moore broke down in very many cases and that some of the most highly toxic fumigants like hydrocyanic acid and ammonia were of very low boiling-point and high volatility. They concluded that chemical constitution or, more precisely, chemical reactivity determined by chemical constitution, was the main determinant of toxic concentration and that physical characters such as volatility merely modify the fundamental reactions. In a later paper Tattersfield (1927) reaffirmed these conclusions, but considered that the modifying influence of physical properties was connected with the varying degree of adsorption of the different compounds by the tissues affected, just as, for example, high-boiling compounds are, in general, more strongly adsorbed by charcoal than low-boiling.

Other investigators (see Shepard, 1939) conclude that the principles of cell permeability are of fundamental importance in insecticide toxicology, believing that enhanced lipoid solubility increases the rate of penetration of the toxic agent, whilst increased molecular size reduces it.

While, as is indicated by Tattersfield & Roberts's results, the boiling-point rule of Holt and Moore is certainly not universally true, it is frequently found to hold over

a limited range within groups of related substances, for example within homologous or substitution series. Within such groups, however, many physical properties of the individual members, such as their vapour pressure or solubility at a fixed temperature, are also correlated with boiling-point, and consequently correlate also with toxicity to insects. Further, such correlations between physical properties and toxicity (toxic concentration), occur not only in fumigant action on insects, but also in the case of groups of substances applied to the most diverse organisms, including aquatic organisms (Ferguson, 1939). Any suggestion, therefore, that the rough boiling-point rule, or some equivalent, is an effect due specifically to the structure of the respiratory system of insects, cannot be correct.

Indeed there is one obvious (but incomplete) explanation which deserves attention. The authors referred to compared the toxicities of fumigants by comparing the values of the molar concentrations in the gas phase which produced a fixed percentage kill of the insects. It is not surprising that, with such a basis of comparison, *some* correlation is found between boiling-point and toxicity. The higher the boiling-point of a substance, the lower is its vapour pressure at a given temperature and thus the lower is the maximum molar concentration of the substance which can possibly be applied as saturated vapour to the insect.

If the boiling-points at atmospheric pressure of a collection of chemical substances, taken at random, are plotted against their vapour pressures at a given temperature, for example 25° C., a close relationship is found—particularly for substances not associated in the vapour phase. The curve so obtained* shows that, on the average, the vapour pressure at 25° C., and consequently the molar concentration of saturated vapour, is about halved for every increment of 17° in boiling-point. The ratio of the molar concentrations of the saturated vapours of two substances boiling 100° C. apart must, therefore, be approximately $1:(\frac{1}{2})^6$ or 64:1. If the vapours of these substances happen to produce the same toxic effect on a given insect species when they are nearly saturated, then, on the above basis of comparison, one would be regarded as sixty-four times more toxic than the other. It is easily seen that this comparison of the external toxic concentrations cannot be a proper comparison of the toxicity of the fumigants. It neglects completely the relative concentrations in the various biophases of the insect. For example, considering any aqueous body fluids of the insect, one substance may be only slightly soluble in such fluids and the other completely miscible. If equilibrium is attained between internal and external concentrations in each case, the internal concentration ratio of the two substances may be quite the reverse of the external ratio. Toxic agents, however, before they act, presumably diffuse from the aqueous body fluid of the insect into, say, a lipoid or other phase. The ratio of the respective concentrations in this phase may again be widely different from what it was in the aqueous and also in the external vapour phase. The relative toxicities of substances as measured by their toxic concentrations

* By plotting the logarithm of the vapour pressure against boiling-point an approximately straight line is produced.

would appear therefore to depend on the phase chosen in which to measure them. Tabulation of the toxic concentrations of fumigants does not by itself give any direct information about what might be called their intrinsic toxicities.

As will become clearer later, such tabulations may often be essentially nothing more than rough tables of the vapour pressures of the substances studied, or, more precisely, of numbers proportional to the vapour pressure values.

It is evident, from what has just been said, that phase-distribution relationships may play a very great part in determining the values of toxic concentrations measured externally to the insect. Some way of eliminating this influence is necessary if valid conclusions are to be drawn from observations of external toxic concentrations about the effect of chemical structure, etc., on toxicity.

A method of eliminating the effect of phase distribution from the results of toxicity measurements in certain cases has been suggested by one of us (Ferguson, 1939). It consists in using as an index of toxicity, not the concentration of the toxic agent measured externally to the organism, but, instead, the thermodynamic activity of the substance at that concentration.

In the case of substances applied in vapour form, the value of the thermodynamic activity at any concentration is very easily obtained. It is numerically equal to the relative saturation of the vapour. Thus, if p_t is the pressure* of the vapour of a substance at which a certain toxic effect is produced, and p_s its saturated vapour pressure at the temperature of the experiment, then the thermodynamic activity at which the substance exercises the specified action is numerically equal to p_t/p_s. Clearly, at a fixed temperature, the thermodynamic activities of all saturated vapours are arbitrarily unity on this basis. The advantage of using thermodynamic activity as the toxic index arises from the fact that, when a substance is distributed in equilibrium between two or more different phases, its thermodynamic activity, suitably defined, has the same numerical value in all the phases.† While therefore we may be, as we usually are, completely ignorant about the nature of that biophase in an organism which is the seat of toxic action, and of the concentration of the toxic agent in that biophase, nevertheless, if a distribution equilibrium has been set up between all the phases concerned, we can state with certainty, from measurements

* p_t (mm.) = concentration in mol./l. $\times 22{\cdot}41 \times \frac{t+273}{273} \times 760$, where $t°$ C. is the temperature of the experiment.

† The more general statement is that the chemical potential of the substance is the same in all the phases in equilibrium. For a definition of chemical potential text-books of chemical thermodynamics should be consulted. The chemical potential, μ, of a substance in a given state (also known as the partial free energy $\bar{F}$), is related to the thermodynamic activity, α, by the equation

$$\mu = \mu° + RT \ln \alpha,$$

where $\mu°$ is constant. For a pure liquid (and hence for a saturated vapour) α is often, as above, conventionally put equal to 1. With this convention, the activities of a substance in different phases in equilibrium with each other are, as stated in the text, equal. Other conventions can be adopted for defining the reference state for the activity. Obviously the same convention must be used for different phases, otherwise the above statement about equality of activities is not true. On the other hand, the equality of μ in different phases in equilibrium is unaffected by the convention used.

of concentration outside the organism, what is the value of the thermodynamic activity at the actual point of attack.

The use of thermodynamic activity as an index of toxicity is claimed to be completely justified only in those cases in which the toxic effect follows the establishment of an equilibrium between the external and internal concentrations. When this happens, it may be assumed, in at least most cases, that the chemical agent is not being used up appreciably in interfering chemically with reaction sequences in the organism, but, as in cases of narcosis, acts as a whole molecule, maintaining its original chemical identity in the body of the organism. The distribution equilibrium is, of course, reversible, but withdrawal of the toxic agent does not necessarily result, as it does in narcosis, in recovery of the organism. Secondary effects may bring about profound derangement, leading perhaps to death. This type of toxic action was classed in the reference just mentioned as 'physical'. It was postulated, following Meyer & Hemmi's treatment of narcosis (1935), that this so-called physical toxicity can be recognized to be operative in a series of related substances, when certain quantitative relationships subsist between the toxic concentrations of the substances measured in a single specified phase and certain of their physical properties. The physical properties in question include vapour pressure, solubility, and coefficients of distribution between immiscible solvents.

When, on the other hand, such relationships do not appear, phase-equilibrium relations are not important factors in determining the value of the external toxic concentrations, and any toxicity observed is classed as 'chemical toxicity'.

The results of measurements reported here of the values of the concentrations of a number of vapours producing 50% kill in samples of grain weevils (the median lethal dose), will be found to exemplify the division of toxic action into two broad classes—chemical and physical—and also the application of the concept of the use of thermodynamic activity as a toxic index. It will be shown that while the toxic concentrations of the vapours of a large number of 'physically toxic' substances exhibit a wide range of values, the corresponding 'toxic thermodynamic activities' exhibit among themselves only relatively slight differences. For physically toxic substances, it is among these relatively small differences in 'toxic activity' between one substance and another that one must seek for any correlation between chemical constitution and toxicity.

Experimental methods

Cultures of grain weevils were made up in $4\frac{1}{2}$ l. sweet jars, 200–300 insects being placed in these on about 3 lb. of seed wheat, previously fumigated for 24 hr. with carbon disulphide to free it from mites and from other insects. Round each culture jar was smeared a ring of banding grease to prevent ingress of mites, etc. The cultures were maintained in an air-conditioned room kept at 25° C., and at about 50% relative humidity. Warm humidified air was continuously blown into the room, which in addition was heated by strip electric heaters controlled by a thermoregulator. The temperature fluctuations as observed on a continuous recorder seldom exceeded

± 1° C. Adult weevils emerged from the cultures in about 42 days. Only insects from 3 to 14 days old from emergence were used for experiment.

Both the fumigation experiments and the breeding of the weevils were conducted in the same room. In each experiment fifty insects were placed in a very small bag of light organdie muslin, which was then suspended from a hook in the top of the fumigation vessel. The fumigation vessels were either 6 l. desiccators or 5 l. bottles fitted with stopcocks attached to the vessel by ground glass joints. A sealed glass bulb containing a weighed amount of the substance to be tested was placed in the fumigation vessel. The vessel was then rapidly evacuated by means of a 'Hyvac' pump. The stopcock was closed and the vessel shaken to break the glass bulb. When the liquid had completely evaporated, which usually required a few minutes, air was admitted to the vessel until atmospheric pressure was re-established, when the stopcock was closed. The vessel was then allowed to stand in the air-conditioned room for 5 hr. After 5 hr., the insects were taken out, and transferred to a glass tube containing grain to serve as food. Counts of the number of dead, living and 'affected' were made after 4 days and after 8 days. In the great majority of cases no insects were 'affected' after 8 days, i.e. they were classed as either 'alive' or 'dead'. When this was not so, counts were continued up to 10 or 12 days, at the end of which the natural mortality came into play as shown by the dying off of the controls. Any insects still described as affected after 12 days were considered to be alive. Results were discarded of all experiments in which the controls showed more than 5% mortality. Control experiments showed that the short period of exposure to vacuum did not affect the resistance of the insects to fumigation.

The volumes of the fumigation vessels were determined by calibration with water. From the known weight of fumigant liberated and the vessel volume, the vapour concentration in milligrams per litre was calculated.

Usually ten to twelve such experiments were performed with each substance, using different concentrations each time. To derive the median lethal dose from the results, use was made of the method of probits devised by Bliss (1935). The logarithms of the doses were plotted against the probits corresponding to each percentage kill. The points lay approximately on two intersecting straight lines as described by Bliss.

The median lethal dose (M.L.D.) was usually obtained by simple inspection of the plot. In a few cases, however (the results for paraffins and monochlorparaffins), the complete statistical method of Bliss was applied to determine the median lethal dose. The accurately calculated M.L.D.'s were not significantly different from those read off the simple plot. For these cases coefficients of variation were also calculated. In the paraffin series the coefficient of variation averaged 4·6%, in the monochlorparaffin series 4·3%. (It may be remarked that any statistical method which assumes a normal distribution of some function of the dose must be applied with care to cases in which the true kill in a given time produced by a saturated vapour is 50%. Obviously the frequency curve here would appear skew.)

The results are presented in the following tables. The tables also show the vapour pressures at 25° C. of the substances used. These were taken from the Landolt-Bornstein Tabellen, or later literature. When no data were available for the vapour pressure, it was calculated from the boiling-point by means of the Ramsay-Young Law or Dühring's method. Such calculated values are shown in brackets. From the median lethal dose expressed in millionths of a gram-molecule per litre has been calculated the pressure of the vapour in millimetres of mercury (p_t) assuming the gas laws to hold. From this there was derived the relative saturations of the vapours giving 50% kill, p_t/p_s, which are tabulated in the fifth column of each table. The sixth column indicates the type of toxicity believed to be operative—*P* represents 'physically toxic', *C* 'chemically toxic'. By *PC* it is to be understood that the toxicity is mainly 'physical', but that to it is added a slight 'chemical' effect.

TABLES OF RESULTS

TABLE 1. *Aliphatic hydrocarbons*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Pentane	511	897	12500	0·45	*P*
Hexane	151	353	4100	0·50	*P*
Heptane	45·6	137	1370	0·56	*P*
Decane	1·6	~12	~85	~1·00	*P*
iso-Pentane	700	1020	14100	0·38	*P*
cyclo-Hexane	97	180	2130	0·41	*P*

TABLE 2. *Aromatic hydrocarbons*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Benzene	93·9	210	2690	0·53	*P*
Toluene	28·5	96	1040	0·68	*P*
Ethyl benzene	9·6	50	470	0·90	*P*
Propyl benzene	3·5	30	250	~1·00	*P*
Butyl benzene	1·2		<50% kill at sat.		*P*
o-Xylene	6·64	31	290	0·82	*P*
p-Xylene	8·87	48	450	0·95	*P*
Mesitylene	2·9	~25	~200	~1·00	*P*
Pseudocumene	1·7		<50% kill at sat.		*P*

TABLE 3. *Monochlorparaffins*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl chloride	4200	166	3280	0·014	*C*
Ethyl chloride	1170	1124	17400	0·28	*P*
Propyl chloride	339	428	5460	0·30	*P*
Butyl chloride	107	200	2160	0·38	*P*
Amyl chloride	32	73	690	0·40	*P*
iso-Propyl chloride	521	740	9330	0·33	*P*
iso-Amyl chloride	(44)	93	870	0·37	*P*
tert.-Butyl chloride	(300)	82	890	0·055	?

TABLE 4. *Alkyl bromides and iodides*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl bromide	1580	3·3	35	0·0004	*C*
Ethyl bromide	465	205	1880	0·075	*C*
Propyl bromide	133?	133	1080	0·15	*C*
Butyl bromide	40	66	480	0·22	*C*
Amyl bromide	13·2	35	230	0·32	*PC*
iso-Propyl bromide	211	516	4220	0·37	*P*
iso-Butyl bromide	60·2	130	940	0·29	*PC*
iso-Amyl bromide	17·9	45	298	0·31	*PC*
Methyl iodide	402	2	14	0·0007	*C*
Ethyl iodide	133	11	71	0·010	*C*
Propyl iodide	44	5·9	34	0·014	*C*
Butyl iodide	14	5·0	27	0·036	*C*
Amyl iodide	4·4	4·6	23	0·10	*CP*
iso-Propyl iodide	67	65	380	0·11	*CP*

TABLE 5. *Halogenated methanes*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl chloride	4200	165	3280	0·014	*C*
Methylene chloride	429	380	4480	0·19	*P*
Chloroform	199	250	2095	0·20	*P*
Carbon tetrachloride	114·5	275	1790	0·29	*P*
Methyl bromide	1580	3·3	35	0·0004	*C*
Methylene bromide	45	90	520	0·20	*P*
Bromoform	5·4	16	64	0·22	*P*
Methyl iodide	402	2·0	14	0·0007	*C*

TABLE 6. *Polychlorparaffins, etc.*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Ethylene dichloride	78	98·9	1000	0·24	*P*
Sym. Tetrachlorethane	6·95	15·4	90	0·24	*P*
As. Tetrachlorethane	14	33·4	199	0·26	*P*
Ethyl chloride	1170	1124	17400	0·28	*P*
β-Trichlorethane	24·5	53	400	0·30	*P*
Ethylidene chloride	225	380	385	0·32	*P*
Methyl chloroform	131·2	290	217	0·31	*P*
Pentachlorethane	4·6	16·3	80	0·32	*P*
Hexachlorethane	0·44		< 50 % kill at sat.		
1:3-Dichloropropane	27	59	520	0·36	*P*
Ethylene dibromide	11·0	0·66	3·5	0·06	*C*

TABLE 7. *Chlorethylenes*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
trans-Dichlorethylene	320	425	4340	0·25	*P*
cis-Dichlorethylene	224	298	3090	0·26	*P*
Trichlorethylene	73	190	1450	0·37	*P*
Perchlorethylene	19	91	550	0·54	*P*

TABLE 8. *Halogenated benzenes*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Fluorbenzene	76	180	1880	0·46	*P*
Chlorbenzene	11·8	45	400	0·63	*P*
Brombenzene	4·2	20	125	0·56	*P*

TABLE 9. *Alcohols*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl alcohol	124·0	100	3130	0·47	*P*
Ethyl alcohol	58·6	85	1850	0·59	*P*
Propyl alcohol	20·1	50	830	0·77	*P*
Butyl alcohol	6·78	~30	~360	~1·00	*P*
Amyl alcohol	2·50		< 50% kill at sat.		*P*
iso-Propyl alcohol	44·4	76	1250	0·52	*P*

TABLE 10. *Ketones*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Acetone	230	110	1900	0·15	*P*
Me Et ketone	93·2	50	700	0·14	*P*
Di Et ketone	36·6	34	400	0·20	*P*
Me Pr ketone	39·0	29	340	0·16	*P*

TABLE 11. *Alkyl formates*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl formate	6245	15	250	0·0007	*C*
Ethyl formate	255	35	480	0·035	*C*
Propyl formate	83	28	320	0·072	*C*
iso-Propyl formate	140	34	390	0·052	*C*

TABLE 12. *Alkyl acetates*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Methyl acetate	210	84	1140	0·10	*P* ?
Ethyl acetate	94	56	640	0·13	*P* ?
Propyl acetate	33	45	440	0·25	*P* ?
Butyl acetate	11·2	41	350	0·58	*P* ?
iso-Propyl acetate	60	90	880	0·27	*P* ?

TABLE 13. *Aldehydes*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol./l.)	p_t/p_s	Type
Acetaldehyde	930	35	796	0·016	*C*
Proprionaldehyde	(350)	25	431	0·023	*C*
Butyraldehyde	110	50	695	0·12	*P* ?
Valeric aldehyde	33	21	245	0·14	*P* ?

TABLE 14. *Amines and carbon disulphide*

Name	V.P. 25° C. (mm.)	M.L.D. (mg./l.)	M.L.D. (10^{-6} g.mol/l.)	p_l/p_s	Type
Ammonia	7500	4·4	260	0·0006	*C*
Diethylamine	250	12	160	0·012	*C*
Triethylamine	66	24	238	0·07	*C*
n-Propylamine	318	8	130	0·007	*C*
n-Dipropylamine	(27)	15	150	0·10	*CP*
n-Butylamine	(110)	20	270	0·05	*C*
iso-Butylamine	(151)	45	620	0·08	*C*
Carbon disulphide	360	30	390	0·02	*C*

DISCUSSION

The relation between toxic concentration and vapour pressure. Examination of the tables shows that the median lethal toxic concentrations (henceforth referred to as the toxic concentrations) of the successive members of groups of related substances, such as homologous or substitution series, in general decrease as their saturated vapour pressures at the temperature of fumigation decrease. Exceptions to this are few. In the three alkyl halide series, the toxic concentrations of the first members, methyl chloride, bromide and iodide, are very much lower than those of nearly all the succeeding members of the series tested, though their vapour pressures are much higher. Similar behaviour is observed in the amine series. A reason for the existence of these exceptions will be discussed later.

In some groups of related substances the rate at which the toxic concentrations of the individual numbers decreases in traversing the group in the direction of decreasing vapour pressure is extremely regular, the toxic concentrations running almost, but, in homologous series certainly, not quite parallel to the vapour pressures. This is illustrated in Figs. 1 and 2, which are logarithmic plots of the measured external toxic concentrations of the members of various series versus their saturated vapour pressures, p_s. The logarithms of the actual toxic concentrations are not plotted but instead the logarithms of the pressures p_l which correspond to the concentrations.

It will be seen that the relation between $\log p_l$ and $\log p_s$ is a linear one. (The reason for drawing lines through only two points in the case of the symmetrical and unsymmetrical ketones is to indicate the rate of change of $\log p_l$ with $\log p_s$ in these cases.)

Each of the lines in Figs. 1 and 2 is, of course, represented by an equation of the type $\log p_l = m \log p_s + \text{constant}$ or $\log p_l = m \log p_s + \log k$, where m and k are coefficients whose value is constant in any one group. It is clear that the relationship between p_l and p_s may also be written in the form

$$p_l = k p_s^m. \qquad (1)$$

The numerical value of k in this equation fixes the position of the line above the

horizontal p_s axis in the logarithmic plot, changes in k moving it bodily up or down in the vertical direction. The value of m determines the slope of the line. While k varies considerably in value between one series and another, m shows little such variation, and for all the homologous series examined, its value appears to lie somewhere between 0·8 and 0·9. In substitution series, as shown in Fig. 2, m appears to be equal to 1·0.

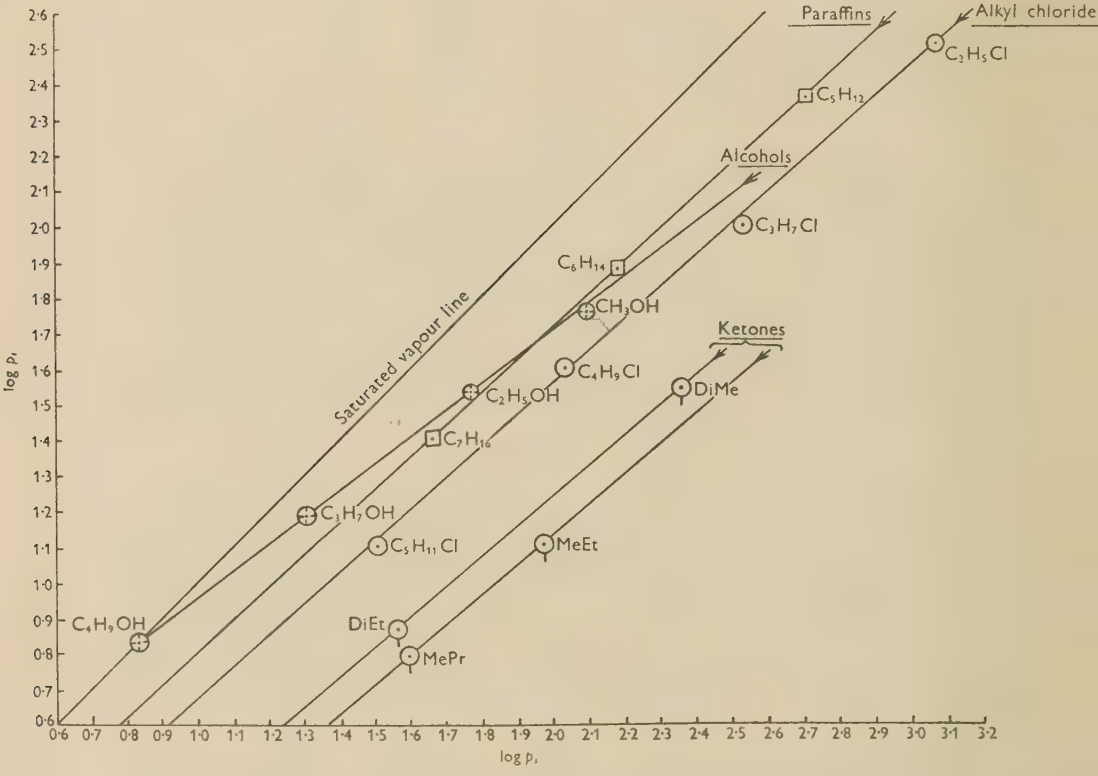

Fig. 1. Toxicity of various homologous series to the grain weevil at 25° C. and 5 hr. exposure.

The type of relation illustrated in Fig 1 and expressible by equation (1) holds for only some of the series examined. These are the paraffins, the alkyl benzenes, the normal alkyl chlorides (excepting methyl chloride), the normal alcohols, the chlorethylenes, certain polychlorethanes and halogen substituted methanes (see Fig. 3), the ketones and possibly certain esters. No such relation is found for the toxic concentrations in the aliphatic amine series, nor with the alkyl formates, the aldehydes, or the alkyl bromides and iodides.

The substances tested in this investigation can therefore be classified into at least two distinct types. For one type relations of the form of equation (1) hold, for the other they do not.

For the reasons given by Ferguson (1939) and briefly summarized in the introduction to this paper, substances of the first type—those which show the relation expressed by equation (1) —are classed as 'physically toxic' substances, and those of the second type as 'chemically toxic'. The latter act in such a way that equilibrium relations have little or no direct influence in determining the value of the externally measured toxic concentration.

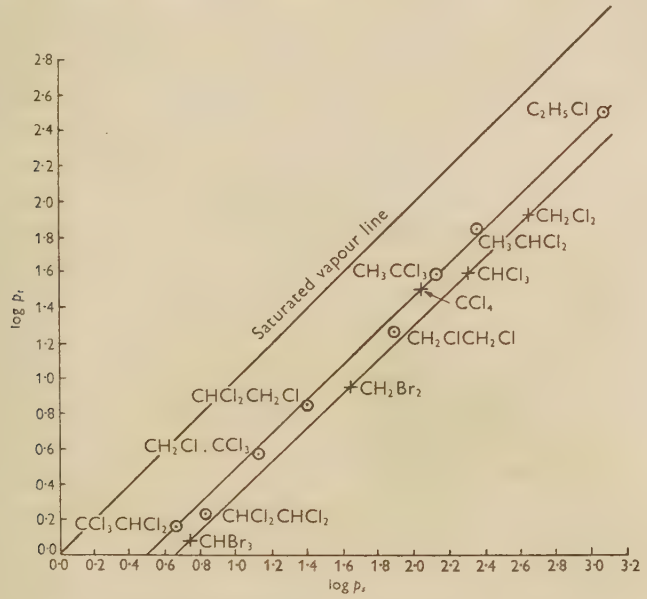

Fig. 2. Toxicity of substitution series to the grain weevil at 25° C. and 5 hr. exposure.

The 'physically toxic' substances are, as can be seen, rather inert chemically. They are in fact typical narcotics. It is probable that their primary action in the organism is of a physical character—as is generally believed to obtain in narcosis. It is believed that all substances are capable of exercising some intensity of toxic action by a physical mechanism. The chemically toxic substances have this physical toxicity added to their chemical toxicity. Often, however, the intensity of the chemical toxicity far outweighs that of the physical action.

Examples of chemical toxicity. The proposition that the 'physically toxic' substances act as whole molecules, and that those classed as 'chemically toxic' have at least undergone reaction in, if not with, the body of the insect is supported by

consideration of the data plotted in Fig. 3. In this figure the logarithms of the toxic pressures of the alkyl chlorides, bromides and iodides are plotted against the logarithms of their saturated vapour pressures at 25° C. It is to be noted that a linear relationship exists for the group ethyl, propyl, butyl and amyl chlorides, which also includes possibly isopropyl and isoamyl chlorides. On the other hand,

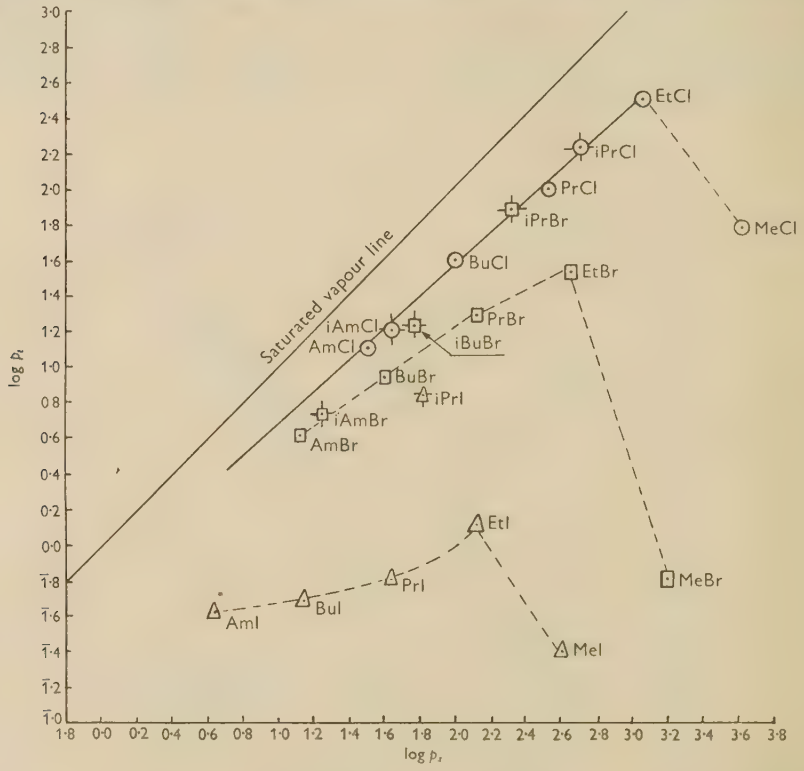

Fig. 3. Toxicity of alkyl halides to the grain weevil at 25° C. and 5 hr. exposure.

all the methyl halides, instead of showing a higher toxic partial pressure than the ethyl compounds, exhibit a very low value. The bromide points do not all lie upon a line, though as the series is ascended they approximate more and more closely to a line passing through amyl-bromide, close to and parallel with the chloride line. The normal iodides do not exhibit a linear relationship, though the plot is consistent with the belief that iodides higher than amyl might behave like the chlorides. A very significant fact is the high toxic concentration of the iso-alkyl bromides and iodides.

The facts illustrated in Fig. 3 are susceptible of a simple explanation, which accords with the known facts regarding chemical reactivity in these series. It may be postulated that, with the exception of methyl chloride, the alkyl chlorides are

physically toxic, that the lower normal bromides in addition to their physical action react chemically, and, that as the length of the hydrocarbon chain increases, the chemical reactivity decreases, tending to disappear at amyl bromide, and that the same behaviour is exhibited by the normal iodides. On the other hand, the *iso*-alkyl bromides and *iso*-propyl iodide, being more stable and less reactive, are only physically toxic.

This explanation is in accord with the results of the very numerous investigations which have been carried out on the reactivity of the alkyl halides. The classic investigations of Menschutkin (1890) were the first of these. Haywood (1922) has summarized the early work up to 1922, and among more recent work may be mentioned that of Conant & Hussey (1925), Grant & Hinshelwood (1933), Winkler & Hinshelwood (1935), Cooper & Hughes (1937). The investigations referred to consisted generally in determining the velocity constants of chemical reactions in which an alkyl halide was one of the reactants. The relative reactivities of the different alkyl halides can be put approximately equal to the ratios of the velocity constants. Though, in the wide range of reactions investigated, considerable quantitative differences were apparent in the relative reactivities, the general results are in striking agreement. First, the reactivities of methyl iodide and methyl bromide (Slator, 1904) are very much greater than those of the corresponding ethyl compounds, and though the reactivities further decrease on ascending the series, the rate of change in reactivity after ethyl is of an entirely different and lower order. In most of the cases quoted by Haywood, for example, methyl iodide is ten to twenty times more reactive than ethyl iodide, while ethyl iodide is only two to three times more reactive than propyl iodide, and propyl iodide is only slightly more reactive than butyl iodide.

Secondly, the iodides are more reactive than the bromides, and the bromides much more reactive than the chlorides. Menschutkin gives the ratio of the reactivities I:Br:Cl as 1:1/6:1/600; Grant & Hinshelwood find the ratios of the velocity constants of hydrolysis by KOH of EtI, EtBr, EtCl at 40° C. to stand in the ratio 1:1/2:1/85.

Thirdly, as has been indicated above, it is generally found that reactivity decreases as a homologous series is ascended, and tends to attain a constant value with the higher members.

Fourthly, the *iso*-alkyl halides are found to be markedly unreactive as compared with the normal compounds. Menschutkin found *n*-propyl iodide to be ten times as reactive as *iso*-propyl iodide, Slator sixteen times. Conant & Hussey found *iso*-propyl chloride to be the most unreactive chloride in the twenty chlorides tested by them, being seventy times less reactive than normal propyl chloride.

The results of the chemical investigations on the reactivity of the alkyl halides are therefore in striking agreement with the facts illustrated in Fig. 3 and reinforce the views expressed on the existence of two types of toxicity—physical and chemical.

Relation between chemical constitution and toxic chemical activities. As explained in the Introduction, for physically toxic substances the ratio p_t/p_s is regarded as the

true measure of toxicity, since it eliminates the irrelevant effect of phase distribution on the data. We shall, therefore, proceed to examine how this index varies with the chemical constitution of substances physically toxic towards grain weevils.

It is to be noted that a low value of the toxic thermodynamic activity corresponds to a high toxicity, just as in the customary mode of expression a low-toxic concentration corresponds to a high toxicity. The maximum value of p_t/p_s is, of course, unity, since p_t may equal, but can never exceed, the saturated vapour pressure.

The fumigants examined in this research and classed in the tables as physically toxic produce 50% kill at thermodynamic activities ranging in numerical value between 0·1 and 1·0. Those classed as chemically toxic, on the other hand, act at much lower values, for example methyl bromide at 0·0004, ammonia at 0·0006, methyl formate at 0·007, acetaldehyde at 0·016. Though it would seem an arbitrary procedure to state that all substances whose activity lies much below 0·1 are chemically toxic to the grain weevil, their vapour pressure relationships suggest that this is indeed the case for the substances examined here. In this connexion it may be pointed out that Gavaudan, Dodé & Poussel (1944) claim that it is possible to fix a lower limit of the threshold biological thermodynamic activity compatible with an action on the lipoids by a purely physical action. According to them, this limit might be expected to be of the order of 0·05. They report results on the effect of a number of substances on mito-inhibition, in which the equi-effective concentrations lie between 0·08 and 1·0. See also the various values of thermodynamic activities of substances exercising various effects (Ferguson, 1939).

The great difference between the use of thermodynamic activity and external concentration as measures of toxicity is well shown in the tables. Thus the toxic values of ethyl chloride and *s*-tetrachlorethane, expressed in terms of activities, are almost identical, being 0·28 and 0·24 respectively. The toxic concentration of ethyl chloride is, on the other hand, almost 200 times greater than that of *s*-tetrachlorethane. A converse example is supplied by ammonia and *o*-xylene, whose median lethal concentrations are practically the same, but whose toxic activities are respectively 0·0006 and 0·82.

Turning now to the effect of chemical constitution on the toxic thermodynamic activity, the first point requiring notice is the increase in toxic thermodynamic activity as a homologous series is ascended, i.e. the decreased toxicity of the higher members. Examples are as follows:

Paraffins	p_t/p_s	Alkyl chlorides	p_t/p_s
C_5H_{12}	0·45	C_2H_5Cl	0·28
C_6H_{14}	0·50	n-C_3H_7Cl	0·30
C_7H_{16}	0·56	n-C_4H_9Cl	0·38
$C_{10}H_{22}$	1·00	n-$C_5H_{11}Cl$	0·40
Alcohols	p_t/p_s	**Alkyl benzenes**	p_t/p_s
CH_3OH	0·49	C_6H_6	0·53
C_2H_5OH	0·59	$CH_3C_6H_5$	0·66
n-C_3H_7OH	0·77	$C_2H_5C_6H_5$	0·89
n-C_4H_9OH	1·00	n-$C_3H_7C_6H_5$	1·00
n-$C_5H_{11}OH$	'Non-toxic'	n-$C_4H_9C_6H_5$	'Non-toxic'

It is obvious that the toxicity *decreases* as a homologous series is ascended, finally becoming zero. This behaviour in homologous series has been often observed but, since toxic concentrations have been used usually as toxic indices, it has been expressed by the statement that toxicity *increases* in a homologous series until a certain member is reached, after which it drops to zero at 'a cut off'. The advantage of the new mode of expression is obvious.

The approach to zero toxicity is well illustrated in Fig. 1 by the slope of the various lines. It will be seen that if sufficiently prolonged they will all cut the saturation line, which is simply a plot of log p_s against itself. Points on the saturation line have $p_t/p_s = 1$.

A measure of the rate of decrease of toxicity is given by the value of the exponent m in equation (1). The values of m found for the various series in this investigation, and also some calculated from the results of other investigators, are given below.

Series	Biological material	Value of m	Source of data
n-Alcohols	Grain weevils	0·79	This paper
	Red spider	0·80	Read (1932)
	*Staph. aureus**	0·82	Tilley & Shaffer (1926)
n-Paraffins	Grain weevils	0·89	This paper
	Mice (narcosis)	0·85	Führer (1921)
Alkyl chlorides	Grain weevils	0·88	This paper
Sym. ketones	Grain weevils	0·85	This paper
Unsym. ketones	Grain weevils	0·88	This paper
Aldehydes	Grain weevils	0·87	This paper

* These data give the bactericidal concentrations of the alcohols in *aqueous solution*. The values of p_t used in calculating m were derived from the known vapour pressures of the alcohols over their aqueous solutions (Butler, Thomson & MacLennan, 1933).

The differences of the m values between different series are not necessarily significant, being perhaps due to experimental error. It is seen that the average value of m lies about 0·85. The fact that the toxicity of the members of homologous series to very different types of organisms not only is expressible by equations of the same form, but that one of the coefficients in the equation is practically the same for different series and different organisms, is significant.

It would seem that the decrease of toxicity in a homologous series is connected with increasing length of the molecule, but, as is suggested by the following table, is not causally connected with the simultaneous decrease of vapour pressure.

Substance	v.p. at 25° C. (mm.)	p_t/p_s	Substance	v.p. at 25° C. (mm.)	p_t/p_s
CH_2Cl_2	429	0·19	$CH_2Cl.CH_2Cl$	78	0·24
$CHCl_3$	199	0·20	$CHCl_2.CHCl_2$	7	0·24
CCl_4	114	0·29	$CH_2Cl.CCl_3$	14	0·26
CH_2Br_2	45	0·20	$CH_2Cl.CH_3$	1170	0·28
$CHBr_3$	5·4	0·20	$CH_2Cl.CHCl_2$	24·5	0·30
			$CCl_3.CH_3$	131	0·31
			$CHCl_2.CH_3$	225	0·32
			$CHCl_2.CCl_3$	4·6	0·32

In the first of these tables it is seen that the halogenated methanes tabulated (with the exception of carbon tetrachloride) are toxic at an almost constant relative

saturation of 0·20 ($m = 1·0$), even though the saturated vapour pressures range from 429 to 6 mm. If this series followed the homologous rule, according to which $p_t = kp_s^m$ with $m = 0·85$, then it is easily calculated that the activity would increase from 0·19 to 0·46 in passing from methylene chloride to bromoform. It is noteworthy that the toxicity defined by thermodynamic activity remains unchanged when chlorine is replaced by bromine in the methane molecule.

In the polychlorethanes three groups can be picked out in which the p_t/p_s values average respectively 0·24, 0·27 and 0·32. The members of each group show a wide range of vapour pressure at 25° C. The differences in the p_t/p_s value for the three groups seem to be connected in some way with the distribution of the chlorine atoms between the two carbons.

Turning now to the influence of substituents such as Cl, OH, OC_2H_5, etc. on the toxicity of the substances listed towards the grain weevil, the following observations may be made:

(1) Substitution of halogen in a hydrocarbon, whether aliphatic or aromatic, has little effect on the toxicity. Thus the p_t/p_s values for benzene and bromobenzene are respectively 0·53 and 0·56; for pentane and amyl chloride 0·46 and 0·40; for successive substitution of chlorine in ethane we have already noticed no difference for certain modes of successive substitution. The calculated value of p_t/p_s for ethane (obtained by extrapolation of the paraffin results) is 0·31. The observed value for ethyl chloride is 0·28. Halogens, as we have already seen, of course sometimes make a molecule 'chemically' toxic and often highly so. In addition to the examples already given in Fig. 3, the low toxic activity of ethylene dibromide may be noted. But, if the action is physical, it does not seem to matter what halogen is substituted—fluorine, chlorine, bromine—all have little effect.

(2) The substitution of hydroxyl in paraffin chains reduces the toxicity. Thus, amyl alcohol is practically non-toxic whilst pentane has a p_t/p_s value of 0·45. Calculated values of p_t/p_s for ethane, propane, and butane obtained by extrapolation and the corresponding values for the alcohols are given below:

C_2H_6	0·31	C_2H_5OH	0·59
C_3H_8	0·36	C_3H_7OH	0·77
C_4H_{10}	0·40	C_4H_9OH	1·0
C_5H_{12}	0·45	$C_5H_{11}OH$	'Non-toxic'

The fact that hydroxyl substitution in the aliphatic paraffins, converting them to polar molecules, reduces toxicity, is of particular interest.

(3) The lower aldehydes are chemically toxic as follows from the run of p_t/p_s values in Table 13. Butyraldehyde and valeraldehyde, however, show the normal rate of decrease of toxic concentration characteristic of a homologous series ($m = 0·87$ from the results of Table 13). It would appear that they are physically toxic. Thus the —CHO group has, in these cases, increased the toxicity of the original hydrocarbons from 0·41 and 0·49 to 0·12 and 0·14 respectively.

(4) The figures for the ketones given in Table 10 show that they are more toxic than the parent hydrocarbon. Thus, methyl propyl ketone has $p_t/p_s = 0{\cdot}16$, and pentane 0·45.

(5) With the exception of the formates, the esters appear to be physically toxic, reasoning from their relatively high p_t/p_s values. The relation between p_t and p_s, however, is not very accurately expressed by equation (1), and the slope factor m is 0·5, much lower than is normal in homologous series. It is possible that the lower members undergo hydrolysis in the insect body to alcohol and acid, and that this complicates the relations observed.

It would be unprofitable at this stage to do more than merely state the above results. Much more data are obviously necessary before any attempt at wider generalization could be made. It is hoped, however, that the data presented furnish a clear proof of the necessity for taking into account distribution relations in general discussions of the relation between chemical constitution and toxic action.

Of course, it is obvious, as Hinshelwood (1946), in discussing anti-bacterial action, has remarked, that 'Partition effects . . . can hardly be invoked to explain the enormous differences in anti-bacterial action of such classes of compound as sulphonamides, acridine derivatives, triphenylmethane dyes, potassium tellurite, and penicillin, to mention only a few of the many specifically inhibitory substances. Such differences depend upon the fact that the drugs in question intervene at particular stages of the reaction sequences involved in growth.' Compared with the many substances which produce intense physiological reactions in minute concentrations, the substances classed here as 'physically toxic' act only at relatively high concentrations. The type of action seems to be closely related to narcosis—a phenomenon, as is well known, which can occur in all forms of life and which is capable of being induced by practically all types of chemical substances (Gavaudan *et al.* 1944). The data and the arguments advanced here may find application in the study of this subject.

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METHODS OF RECORDING APHID POPULATIONS FOR USE IN RESEARCH ON POTATO VIRUS DISEASES

By L. BROADBENT
Rothamsted Experimental Station, Harpenden, Herts

(With 8 Text-figures)

Methods of counting aphids infesting potato crops are reviewed and discussed. The results of an aphid survey in different parts of England during 1946 and of counts at Rothamsted during 1947 are given and used to illustrate methods of expressing the counts; the extent of virus spread in 1946 showed no close correlation with aphid numbers. It is concluded that an estimate of the number of aphids per plant is better for virus disease research than the number per 100 leaves. A method of estimating the number of aphids per plant with tolerable accuracy is described, but it is concluded that for most surveys a method which does not necessitate the counting of the aphids is adequate. Such a method, based on the percentage of leaves infested, is described.

The discovery that potato viruses are transmitted by aphids (Botjes, 1920; Schultz & Folsom, 1923; Murphy, 1923), stimulated interest in the spread of virus diseases in the field, especially in areas considered suitable for the production of high-grade seed. Some of the most intensive studies were those by Maldwyn Davies and his co-workers in North Wales, as a result of which Davies (1934) came to the conclusion that the greatest potential danger to a crop in a seed area was from the *Myzus persicae* Sulz. arriving from without the crop, and the degree to which these aphids moved from plant to plant. Normally, the main migration to potatoes in North Wales was in early June, and these alatae continued to move from plant to plant for about a month. By early July the migration has usually ceased, and Davies considered that the figure which gave some indication of the number and movement of the alatae would be obtained by an estimate of the mid-July infestation. This mid-July estimate he termed the 'index', which he obtained by counting the number of aphids per 100 random lower leaves as the crop was traversed. A number of counts made over the same area gave comparable results. In seed-producing districts the index figure was generally well below 100 and usually below 20. He stressed that a high maximum infestation at a later date need not be indicative of a large spread of virus disease, because it may represent only the amount of reproduction of aphids that has occurred within the crop. If the crop is originally healthy a rapid increase of apterous aphids within the field would not be followed by a spread of virus diseases. He considered that, when the arrival of alatae is delayed because of unfavourable weather conditions, the mid-July index would still indicate the intensity of the migration, for even in the latest seasons the main migration of alatae would have been completed by early July. The mid-July figure would be misleading only in an early season, when the peak may have been passed.

This work of Davies has formed the basis of most of the surveys made in different parts of the world. The method was used for many years in North Wales (Whitehead, 1943), in Scotland (Davies, 1939), in Denmark (Hansen, 1941), in south-west England (Staniland, 1943) and in Derbyshire (Broadbent, 1946). Joyce (1938) and Thomas & Jacob (1943), continuing Davies's work in North Wales, modified his method by taking fifty plants at random and selecting an upper, a middle and a lower leaf from each. They still expressed the count of aphids as an index per 100 leaves. Simpson (1940) in Maine, U.S.A., used the same method. Norris & Bald (1942) in Australia took 100 leaves alternately from upper, middle and lower positions in the plants, but expressed the aphid count finally as number per forty-eight leaves. Jacob (1944), making a survey in Cumberland, Durham, Northumberland and Westmorland, again occasionally took three leaves per plant, but usually made counts on 100 leaves taken at random from the middle parts of 100 plants. The two methods gave similar results when the data were reduced to a common basis of number of aphids per 100 leaves. Loughnane (1940), making diagonal traverses of Irish fields, chose plants at random and counted the aphids on five or six leaves per plant in different positions on the plant, the total number of leaves taken varying with the size of the crop. The counts were again expressed as aphids per 100 leaves, as they were by Doncaster & Gregory (1948) who, choosing plants at random in different parts of the field, examined every third leaf, starting with the lowest, on the main shoot only on each plant.

Much of the survey work has been undertaken to determine the critical aphid populations above which the maintenance of healthy stocks of seed potatoes is not a practicable proposition. At the same time many workers have made counts of the aphids and other insects present throughout the period of growth of the potatoes to determine the life cycles of the insects, the biological inter-relations of the species present, the effects of different weather conditions upon them and their relation to the spread of virus diseases at different times of the season. Heinze & Profft (1938, 1940) considered Davies's and similar methods unsuitable for many of the above studies because, while they allow the degree of infestation on a portion of the field to be approximately ascertained, they in no way give an exact picture of conditions, especially concerning the movement of aphids on individual plants, during the growth period of the plant. In their work in Germany, they chose at random twenty to thirty observation plants in each field, and, leaf by leaf, counted the total insect fauna once or twice weekly. Moericke (1941), also in Germany, studied the populations by beating the plants to shake off the insects, which were taken to the laboratory for examination. Like Heinze & Profft, he recorded the population as aphids per plant. Thomas & Jacob (1943) tried a beating method of sampling, but found it to be unsatisfactory, as not all the aphids could be dislodged. Bald, Norris & Helson (1946) concluded that the number of aphids per plant was the more logical basis for estimating whether aphids had any preference for different varieties of potatoes. They estimated the populations by counting the aphids on

twelve leaves of each variety and multiplying the number by the ratio between the total leaf area of the twelve leaves and the total leaf area of the twelve plants from which they were picked. This figure, divided by twelve, gave the number per plant.

Aphid survey, 1946

Experimental plots of Majestic potatoes, with healthy seed and virus-infected tubers planted according to a standard plan, were grown in different parts of the country in 1946 (Broadbent & Gregory, 1948). All the records of virus disease quoted were made by Dr P. H. Gregory. Miss B. N. Hawkey kindly made four of the five aphid counts at Slough. In addition to the counts and trap catches made on the experimental fields, counts were made on commercial fields at Spalding, Lincs, and at seven places in the Peak District of Derbyshire. Half-hundredweight lots of seed were obtained from some centres and the percentages of leaf roll and rugose mosaic in these samples are given in Table 2. Data from the experimental fields are also given. Doncaster & Gregory (1948) have shown that the spread of virus disease is usually local, the chances of infection falling rapidly with increasing distance from the infector: thus most of the infections in the five plants nearest to the infectors in the experiments will have come from the infectors.

Table 1 gives the numbers of *Myzus persicae* (Sulz.) counted by taking at random thirty-three plants and taking an upper, a middle and a lower leaf from each, plus one leaf from a thirty-fourth plant. *Aulacorthum solani* (Kalt.) was nowhere numerous, *Macrosiphum solanifolii* (Ashm.) increased to over 100 per plant only at Spalding (573 per plant) and Sutton Bonington (172 per plant), while *Aphis rhamni* Fonsc. was numerous only at Oaker, Ashover, Birchover and Monyash, all Derbyshire centres in the Matlock area, where *A. rhamni* also occurred in 1945. A maximum of ninety *A. rhamni* per plant occurred at Baston, near the only site of *Rhamnus cathartica* known in the Spalding area, whereas this aphid was virtually absent from the other two Lincolnshire fields. Apparently in 1946 this aphid occurred only in close proximity to its winter host.

Also in Table 1 are given the percentage of leaves examined which were infested with *Myzus persicae*, the percentage of groups of three leaves (upper, middle, lower) which were infested, the estimated number of leaves per plant (based on counts on ten plants, two random groups of five), and the estimated number of *M. persicae* per plant calculated from the count per 100 leaves and the number of leaves per plant.

At Rothamsted, Slough, Spalding and Sutton Bonington, the peak of the infestation was before or about the middle of July. At Baston and Postland, only a few miles from Spalding, the peak was towards the end of July, and at all the Derbyshire centres it was in the second half of August. Davies (1939), Hansen (1941) and many other workers have found earlier and bigger infestations near towns or other suitable overwintering sites for *M. persicae*, such as market gardens. The centres with early peaks in 1946 were in such areas. Very few *M. persicae*

TABLE 1. *Observations on potato fields*, 1946

Centre and planting date	Date	*M. persicae* per 100 leaves Apterae-Alatae Ad: Nym-Ad: Nym*	Leaves per plant	*M. persicae* Leaves infested (%)	*M. persicae* Groups of 3 leaves infested (%)	*M. persicae* Estimated no. per plant
Rothamsted,	8. vi	3 : 6–2 : 0	9	8	21	1
Herts	14. vi	2 : 7–1 : 0	21	7	18	2
17. iv. 46	27. vi	8 : 25–1 : 0	34	21	48	11
	11. vii	12 : 61–0 : 4	65	36	81	50
	25. vii	1 : 12–0 : 0	65	7	18	9
	9. viii	3 : 11–0 : 0	76	6	18	11
	24. viii	5 : 10–0 : 1	79	8	21	13
Slough,	13. vi	40 : 154–5 : 1	13	48	81	26
Bucks	27. vi	1 : 131–2 : 0	45	50	90	60
30. iv. 46	11. vii	7 : 22–0 : 0	71	20	54	20
	25. vii	6 : 11–0 : 0	89	12	30	15
	8. viii	0 : 0–0 : 0	90	0	0	0
Baston,	5. vi	3 : 0–0 : 0	9	1	3	0·3
Lincs	19. vi	0 : 1–0 : 0	26	1	3	0·3
16. iv. 46	19. vi	0 : 1–0 : 0	—	1	3	0·3
	3. vii	8 : 62–0 : 10	37	10	21	30
	16. vii	29 : 217–2 : 0	63	51	90	157
	29. vii	41 : 241–2 : 130	75	55	100	311
	15. viii	23 : 114–0 : 23	98	61	100	157
	25. viii	0 : 6–1 : 0	100	5	15	7
Postland,	5. vi	0 : 0–0 : 0	7	0	0	0
Lincs	19. vi	2 : 9–1 : 0	15	5	15	2
24. iv. 46	3. vii	5 : 45–1 : 0	26	19	45	13
	16. vii	4 : 105–0 : 0	43	43	72	47
	29. vii	19 : 157–4 : 70	80	49	100	200
	15. viii	5 : 26–1 : 3	138	26	60	48
	25. viii	1 : 4–0 : 0	150	4	3	8
Spalding,	19. vi	0 : 6–0 : 0	20	3	9	1
Lincs	4. vii	10 : 89–1 : 0	38	28	60	38
16. iv. 46	16. vii	58 : 526–4 : 16	70	60	100	423
	29. vii	12 : 172–8 : 108	96	56	89	289
	15. viii	0 : 3–0 : 0	106	2	6	3
	25. viii	1 : 4–0 : 0	90	4	12	5
Sutton	17. vi	2 : 7–0 : 0	30	7	21	3
Bonington,	1. vii	4 : 42–1 : 0	65	22	57	30
Notts	18. vii	60 : 704–2 : 186	160	74	100	1523
15. iv. 46	31. vii	12 : 132–2 : 100	200	74	94	492
	17. viii	1 : 6–0 : 0	201	6	12	14
	27. viii	2 : 4–0 : 0	150	5	15	9
Ashover,	1. vii	0 : 3–2 : 0	5	3	9	0·3
Derby	18. vii	1 : 7–0 : 1	24	8	21	2
4. v. 46	30. vii	2 : 15–1 : 0	59	11	30	11
	25. viii	56 : 256–0 : 58	80	73	96	296
	19. ix	10 : 9–0 : 56	3	—	—	2

* Aphid counts are expressed as: adult apterae : nymphs of apterae-adult alatae : nymphs of alatae (Ad: Nym-Ad: Nym). The first two instars of nymphs of alatae would be classified as nymphs of apterae because the wing buds are not visible in the field.

TABLE 1 (*continued*)

Centre and planting date	Date	*M. persicae* per 100 leaves Apterae-Alatae Ad:Nym-Ad:Nym	Leaves per plant	*M. persicae* Leaves infested (%)	*M. persicae* Groups of 3 leaves infested (%)	*M. persicae* Estimated no. per plant
Birchover,	2. vii	0 : 6–1 : 0	10	3	9	1
Derby	18. vii	3 : 27–0 : 0	24	17	48	7
16. v. 46	30. vii	3 : 14–0 : 0	69	12	30	12
	27. viii	22 : 90–0 : 40	102	54	84	155
	19. ix	13 : 52–0 : 45	21	51	81	23
Bradwell,	2. vii	1 : 1–0 : 0	6	1	3	0·1
Derby	19. vii	8 : 25–0 : 0	25	14	33	8
13. v. 46	30. vii	3 : 32–0 : 0	81	11	24	28
	26. viii	56 : 310–2 : 26	81	72	100	319
	19. ix	*c.* 50	6	—	—	3
Bretton,	2. vii	0 : 0–0 : 0	6	0	0	0
Derby	17. vii	1 : 9–0 : 0	25	9	18	3
16. v. 46	30. vii	0 : 9–1 : 0	56	9	28	6
	16. viii	4 : 15–0 : 5	79	16	39	19
	26. viii	11 : 32–1 : 6	73	31	63	37
	20. ix	3 : 18–0 : 11	64	20	54	21
Edale,	19. vii	3 : 11–0 : 0	15	6	12	2
Derby	16. viii	5 : 13–0 : 2	29	12	27	6
27. v. 46	26. viii	7 : 56–1 : 2	44	28	57	29
	20. ix	0 : 0–0 : 0	50	0	0	0
Hathersage,	2. vii	1 : 0–0 : 0	39	1	3	0·4
Derby	17. vii	3 : 13–1 : 0	28	11	33	5
28. iv. 46	31. vii	8 : 24–0 : 3	63	20	54	22
	26. viii	15 : 69–1 : 21	89	42	78	94
	19. ix	6 : 47–0 : 21	27	37	75	20
Kinder,	31. vii	0 : 6–0 : 0	23	5	15	1
Derby	16. viii	14 : 46–1 : 4	46	37	69	30
25. v. 46	26. viii	14 : 55–0 : 9	39	35	72	30
Monyash,	19. vii	0 : 8–0 : 0	21	6	18	2
Derby	31. vii	4 : 25–0 : 0	55	15	42	16
25. v. 46	16. viii	27 : 143–2 : 46	69	67	100	150
	26. viii	44 : 240–0 : 74	80	80	100	286
	19. ix	*c.* 75	6	—	—	5
Oaker,	6. vi	0 : 6–3 : 0	5	6	18	0·4
Derby	17. vi	2 : 3–2 : 0	21	5	15	1
16. v. 46	3. vii	1 : 5–0 : 0	39	6	18	2
	17. vii	6 : 21–0 : 0	62	20	51	17
	30. vii	6 : 24–1 : 4	132	19	48	46
	16. viii	7 : 92–0 : 8	183	41	75	196
	27. viii	4 : 26–0 : 4	89	21	45	30
	19. ix	0 : 0–0 : 0	5	0	0	0

were found overwintering in the Peak District of Derbyshire in the winter 1945–6, compared with those in Lincolnshire, Hertfordshire and Bedfordshire.

A large proportion of the season's spread of virus was the result of the activity of the early migrants (Broadbent & Gregory, 1948), but as Table 2 shows, a mid-

July index count bore little relation to the spread of virus disease. At Rothamsted, Slough and all the Derbyshire centres, *M. persicae* was below 100 per 100 leaves, yet the spread of virus was considerable at Rothamsted and appreciable at all the other centres. Both leaf roll and rugose mosaic each spread to about the same extent at Rothamsted and Sutton Bonington, but at Sutton Bonington there were 1523 *M. persicae* per plant in mid-July, compared with fifty at Rothamsted. At both centres these were also the maximum counts.

TABLE 2. *Mid-July counts of* Myzus persicae *and virus spread in* 1946

Centre	Potato variety and health	*M. persicae* Per 100 leaves	*M. persicae* Per plant	Virus spread experiment – % virus in 5 plants each side of infectors: Leaf roll	Virus spread experiment – % virus in 5 plants each side of infectors: Rugose mosaic
Rothamsted, Herts	Majestic S.S.	77	50	29·4	17·9
Slough, Bucks	Majestic S.S.	29	21	9·4	6·8
Baston, Lincs	Majestic S.S.	248	157	32·2	9·2
Postland, Lincs	Majestic S.S.	109	47	38·7	7·8
Sutton Bonington, Notts	Majestic S.S.	952	1523	31·7	25·6
Bretton, Derby	Majestic S.S.	10	3	5·8	1·0
Oaker, Derby	Majestic S.S.	27	17	13·2	10·6

				Percentage virus in crop samples from commercial fields: 1946 L.R.	1946 R.M.	1947 L.R.	1947 R.M.	L.R. spread
Spalding, Lincs	Majestic S.S.	604	423	0·5	0	6·4	0	× 12·8
Ashover, Derby	Red King S.S.	9	2	1·0	0	2·8	0	× 2·8
Birchover, Derby	K. Edward S.S.	30	7	4·3	0	18·3	0	× 4·2
	Red King S.S.	30	7	0·5	0	1·4	0	× 2·8
Bradwell, Derby	Red King S.S.	33	8	2·0	0	12·5	0	× 6·2*
Edale, Derby	Red King S.S.	14	2	—	—	—	—	—
Hathersage, Derby	K. Edward A	17	5	—	—	—	—	—
Kinder, Derby	Majestic A	0	0	—	—	—	—	—
Monyash, Derby	K. Edward A	8	2	3·2	0	8·1	0	× 2·5

* At Bradwell there was a crop of once-grown King Edward potatoes on the other side of a narrow road, with 90% leaf roll and 0·5% rugose mosaic in 1946, further evidence of the large spread of leaf roll in Derbyshire in 1945 (see Broadbent, 1946).

Fig. 1, showing the growth curves of the plants from the same batch of seed at five widely separated centres, emphasizes that site and planting date greatly affect the development of the crop, and that 100 leaves at any arbitrary date give very different proportions of the crops at different centres. It is necessary, therefore, in comparing aphid infestations at different centres, to consider the stage of development of the plant and record the population per plant rather than per 100 leaves.

In Table 2 are given the counts recorded by the two methods at the date suggested by Davies, mid-July. The count per 100 leaves at Sutton Bonington was 1·6 times that at Spalding, whereas the population per plant was 3·6 times as great; the count

at Postland was 1·4 times that at Rothamsted, whereas the population per plant was 0·9 times. An operation which affects both methods and may lead to an underestimate of early aphid population is 'earthing up', which may bury some of the lower leaves.

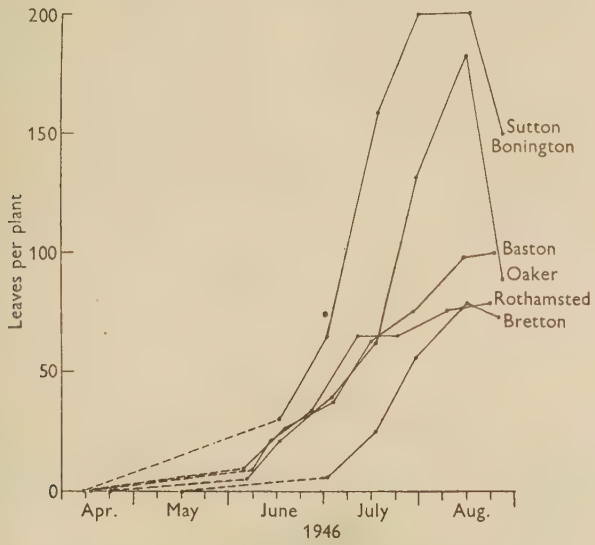

Fig. 1. Influence of site and planting date on growth of potato crop. All crops from the same batch of seed.

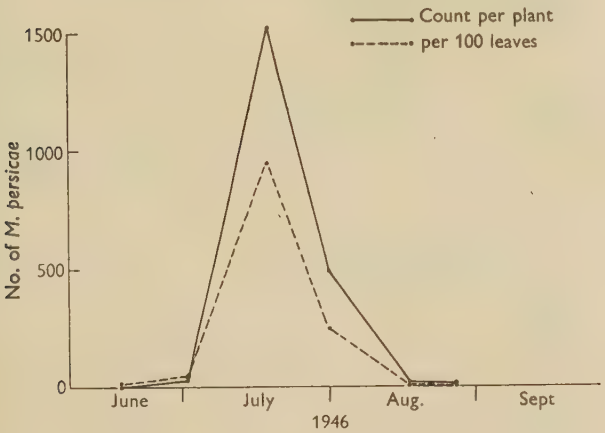

Fig. 2. The infestation of potatoes by *M. persicae* at Sutton Bonington.

Figs. 2–6 show the different ways in which population curves may be affected by the method of expressing the count. Where the count is expressed as number

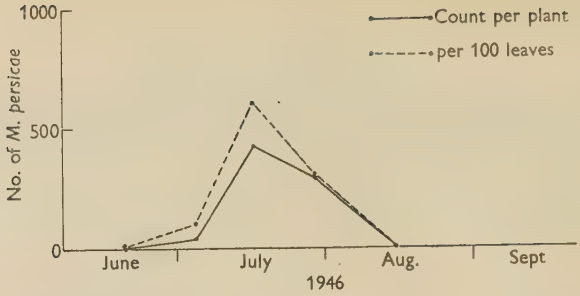

Fig. 3. The infestation of potatoes by *M. persicae* at Spalding.

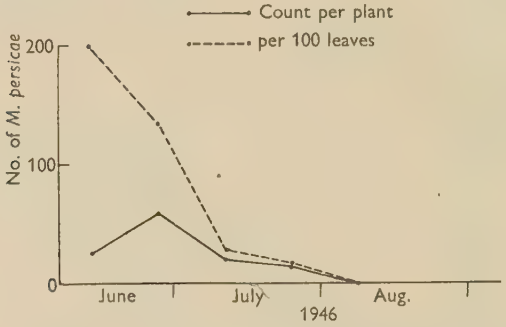

Fig. 4. The infestation of potatoes by *M. persicae* at Slough.

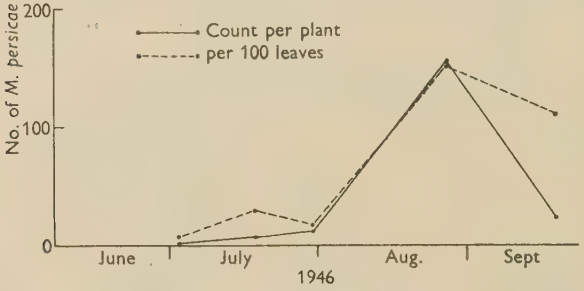

Fig. 5. The infestation of potatoes by *M. persicae* at Birchover.

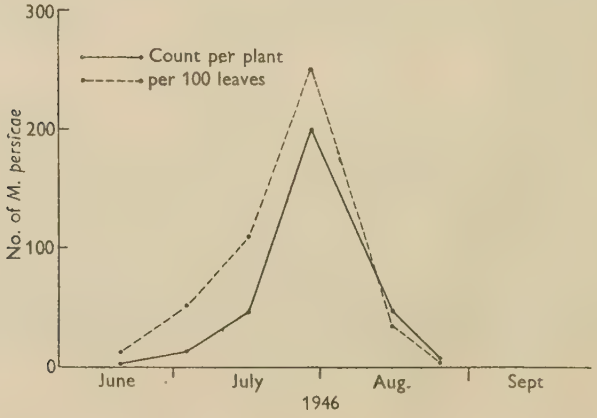

Fig. 6. The infestation of potatoes by *M. persicae* at Postland.

per 100 leaves the peak of the infestation may be lower than if the count is expressed as number per plant, as at Sutton Bonington (Fig. 2); or higher, as at Spalding (Fig. 3); or the peak may be very early in the season and the early counts much higher, as at Slough (Fig. 4); or the later counts may be higher as the foliage dies, as at Birchover (Fig. 5). At every centre in 1946 the early figures by the 100-leaf method were higher and the increase in population to the peak was less rapid than when aphids were recorded per plant, e.g. Postland (Fig. 6).

Aphid counts in 1947

Counts of aphids were made at frequent intervals throughout the season on two varieties of potatoes at Rothamsted. A 0·4 acre plot of the variety Arran Pilot was situated in a field of variety Majestic; the aphid counts on the Majestic plants were made on an area adjoining the Arran Pilot. To obtain a better estimate of leaves per plant, the leaves on four groups of five plants were counted, i.e. on twenty plants. The counts of *M. persicae* are given in Table 3. *M. solanifolii* and *Aulacorthum solani* were not numerous, but large colonies of *Aphis rhamni* developed in late July and early August. Braconid parasites were present in small numbers late in the season. Predators, scarce in 1946, were numerous in 1947, Syrphids in particular being present throughout the season. Syrphid larvae also reduced the colonies of *Myzus persicae* developing on outdoor peaches and nectarines at Rothamsted, and few alate spring migrants were produced. A small number of *M. persicae* overwintered and alatae were produced on an ornamental almond tree (probably a hybrid between *Prunus amygdalus* and *P. persicae*) in Harpenden. No *Myzus persicae* were found overwintering as viviparae on outdoor crops or in mangold clamps in the area.

The scarcity of nearby overwintering *M. persicae* was reflected in the low populations on both Majestic and Arran Pilot potatoes during June. In the middle of July the populations suddenly increased, as the result of an invasion of alatae. There was circumstantial evidence that these came from sugar beet. Trapping records indicated that the invasion took place during 15–16 July. During the dry, hot summer the aphid population increased rapidly. As the counts (Table 3) and traps (Table 4) indicated, the summer dispersal migration from the potatoes took place during the last few days of July and the first fortnight in August, after which the plants remained relatively free until the middle of September. A count on the Majestic potatoes on 1 October showed an increase, whether by multiplication of the few previously present or by a fresh invasion of alatae from another crop is unknown. Frequent counts on the potatoes were not made at this time and there is nothing to indicate whether the alatae caught on the traps in mid-September were bred on the crop, or were coming from outside. Doncaster & Gregory (1948) concluded that the absence of a large number of young nymphs after the summer dispersal was evidence to support the view that the newly developed alatae left the crop without flying from plant to plant. They stated that a high proportion of alatae was never found on the crop at that time. In 1947 0·5% of the count on the

TABLE 3

Date	No. of leaves examined	*M. persicae* per 100 leaves Apterae-Alatae Ad : Nym-Ad : Nym	Leaves per plant	Leaves infested (%)	*M. persicae* Groups of 3 leaves infested (%)	*M. persicae* Est. no. per plant	*M. persicae* Est. no. per plant assuming a 5 : 3 : 2 leaf ratio
Observations on Majestic potatoes, Rothamsted, 1947, taking upper, middle and lower leaves per plant							
5. vi	100	0 : 0– 0 : 0	—	0	0	0	—
9. vi	200	0 : 0– 0 : 0	—	0	0	0	—
16. vi	300	0·3 : 1– 0 : 0	16	0·3	1	0·2	—
23. vi	400	0 : 0– 0 : 0	20	0	0	0	—
2. vii	200	1 : 7– 0 : 0	53	4	8	4	—
5. vii	300	1 : 3– 1 : 0	57	3	6	3	—
14. vii	200	7 : 35– 1 : 1	76	18	36	33	27
21. vii	78	18 : 421–22 : 38	106	82	100	529	406
1. viii	100	95 : 628– 4 : 87	157	88	100	1278	1137
6. viii	100	61 : 355– 8 : 85	179	74	100	911	752
14. viii	48	6 : 54– 0 : 2	164	37	81	102	102
27. viii	100	5 : 17– 0 : 0	141	13	27	31	—
8. ix	100	5 : 27– 0 : 0	—	9	—	—	—
1. x	100	44 : 163– 2 : 173	—	59	—	—	—
Counts per 100 leaves, taking every tenth leaf on the plants							
22. vii	405	16 : 274–17 : 37	154	68	—	530	—
6. viii	209	40 : 346– 2 : 76	206	70	—	956	—
Observations on Arran Pilot potatoes, Rothamsted, 1947, taking upper, middle and lower leaves per plant							
16. vi	200	0·5 : 1– 0 : 0	12	1	3	0·2	—
23. vi	150	0 : 1– 0 : 0	19	1	2	0·1	—
2. vii	100	1 : 9– 0 : 0	41	3	9	4	—
15. vii	100	26 : 131– 2 : 14	55	45	78	95	78
23. vii	100	52 : 608–19 : 154	64	84	96	533	459
30. vii	100	200 : 1742– 8 : 353	87	97	100	2004	1589
7. viii	100	52 : 449– 3 : 92	110	84	96	656	592
14. viii	52	1 : 15– 0 : 0	95	25	47	15	15

TABLE 4. *Mean catches per trap of* Myzus persicae *on traps in the Rothamsted potato field,* 1947

Date	No. of days	No. of *M. persicae*
5–26. vi	21	0
26. vi.–7. vii	11	0·25
10–14. vii	4	1·5
14–18. vii	4	62·25
18–22. vii	4	1·5
22–25. vii	3	1·25
25–27. vii	2	2·75
27–29. vii	2	8·5
29. vii–1. viii	3	10·75
1–4. viii	3	13·75
4–8. viii	4	19·25
8–14. viii	6	10·5
14–18. viii	4	1·0
18–25. viii	7	0·5
25. viii–1. ix	7	0
1–8. ix	7	0·5
8–15. ix	7	4·5
15–22. ix	7	4·5
22–29. ix	7	3·0
29. ix.–6. x	7	1·0

Majestic potatoes were alatae on 1 August, 1·6% on 6 August; on Arran Pilot potatoes 0·3% were alatae on 30 July, 0·5% on 7 August. These facts, and the small number of nymphs a week later (Table 3), support the above view. On the other hand, alatae were often observed flying from plant to plant, and on 7 August an alate *M. persicae* was seen depositing nymphs on an Arran Pilot plant, and on 1 October on a Majestic plant. Thus although most alatae may leave the potato crop when they are ready to fly, some, or others coming from outside, fly from plant to plant and could be transmitting virus diseases.

The large dispersal from other summer hosts at Rothamsted in 1947, to potatoes late in the season, shows that the main migration to potatoes cannot be relied upon to occur by early July, as was assumed by Davies. The late 1947 migration could easily have been overlooked if counts had not been made at frequent intervals because the incoming alatae arrived at the same time as alatae were developing in small numbers on the potatoes. Such a large invasion will presumably occur rarely, but smaller numbers arriving from other crops might account for the late peaks of infestation in all the Derbyshire fields in 1946. They may also account in part for the late development of the infestation noted by Davies (1939) to be characteristic of good seed-producing areas, which are never near areas where *M. persicae* can overwinter easily.

Comparison of methods of estimating the population of *Myzus persicae*

The counts of *M. persicae* on the Majestic and Arran Pilot potatoes are given in Table 3 as number per 100 leaves and as estimated per plant. Had an index count been made in mid-July, the figures would have been 44 per 100 leaves on the Majestic and 173 per 100 leaves on the Arran Pilot potatoes, both counts bearing little relation to the populations present in the next few days. Even if counts had been made at monthly intervals, mid-June, mid-July and mid-August, as has been done in some surveys, the results would not have shown the large population which developed in late July, i.e.

	mid-June	mid-July	mid-August	
Majestic	1·3	44	62	Per 100 leaves
Arran Pilot	1·5	173	16	Per 100 leaves

If the populations are compared at their peak, the Arran Pilot supported an infestation 2·8 times as great as that on the Majestic potatoes, as shown by counts on 100 leaves, and only 1·6 as great if estimated per plant (Fig. 7). Twice during the season, counts were made on the Majestic potatoes by taking every tenth leaf on the plant and recording the insect population present, the length of the leaf and its position on the plant (see following paper). Below, the counts made by the different methods on 21–23 July, a week after the invasion by alatae, are compared:

Count ...	3 leaves per plant		Every tenth leaf	
	Per 100 leaves	Per plant	Per 100 leaves	Per plant
Majestic	499	529	344	530
Arran Pilot	833	533	—	—

The apparent large difference in infestation on the two varieties when the count is expressed as number per 100 leaves is seen to be an effect created largely as a result of the differences in size of plants. In fact, the two varieties were infested to a similar degree, which is what might be expected if the alatae were distributed at random over the field and there was no varietal preference.

When counts were made on every tenth leaf of the plants, it was evident that large numbers of *M. persicae* were present on even the smallest leaves and on the flowers. This is not a common phenomenon, *M. persicae* usually preferring the lower, older leaves. In 1946 and 1947 the 100-leaf counts were made on leaves over 1 in. long, and small leaves, including the apical rosette, were ignored when the number of leaves per plant was counted. While making counts on every tenth leaf, every leaf, however small, was included, with the result that an average of 154 leaves per plant were counted on 22 July, compared with 106 per plant estimated by counts on twenty plants. While the average number of *M. persicae* per plant was the same by the two methods, it would be more satisfactory in future to count all leaves and to take a proportion of small ones in examining them for aphids.

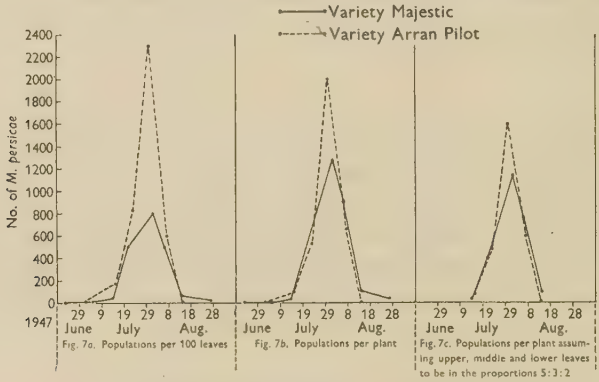

Fig. 7. *M. persicae* on potatoes. Rothamsted, 1947.

THE PROPORTION OF LEAVES AND APHIDS AT DIFFERENT HEIGHTS

To obtain an estimate of leaves of different sizes in the upper, middle and lower parts of the plants, four plants were examined on 7 August. The counts were made on Majestic plants, two planted early in May and two late in May in a date-of-planting trial. Although the sample was small, upper, middle and lower leaves occurred in the ratio 5:3:2 on the average; leaves under 3, 3–6, and over 6 in. long were in the ratio 3:5:2. Similar ratios were found when counts were made on every tenth leaf on the Majestic potatoes. These ratios undoubtedly vary early and late in the season and in plants grown under different conditions, but assuming that the upper, lower and middle leaves were in the ratio 5:3:2, the mid-season counts have been recalculated (Table 3) and Fig. 7*c* shows that on this basis the infestations on

the two varieties were even closer than previously estimated, the Arran Pilot potatoes having 1·4 times the population on the Majestic at the peak. Clearly, leaves in the different parts ought to be chosen according to the ratio in which they occur if the aphids are equally distributed over the plant, or if unequal distribution makes advisable more counts on the more densely populated part (see following paper), then such counts ought to be adjusted to the appropriate ratio.

Suggested counting methods

The data presented above show that estimates of the number of aphids infesting a potato crop vary with the method of expressing the results. For virus studies the number of aphids per plant is perhaps the most useful estimate, and the following method is suggested, as giving a reliable result:

From each of forty random plants in a traverse of the field take one upper, one middle and one lower leaf, taking appropriate numbers of small, medium and large leaves. Count all the leaves on alternate plants of the forty chosen, classifying them as upper, middle or lower. From these data the average population per leaf at each level is easily calculated and then is multiplied by the number of leaves at each level. The sum of the three products is the population per plant.

Example: Suppose the count on 40 plants was:

On 40 upper leaves 20 aphids = 0·5 per leaf
On 40 middle leaves 80 aphids = 2·0 per leaf
On 40 lower leaves 100 aphids = 2·5 per leaf

Suppose the average number of leaves on twenty plants was upper leaves, 100; middle leaves, 60; lower leaves, 40. Then the number of aphids per plant

$$= (100 \times 0{\cdot}5) + (60 \times 2{\cdot}0) + (40 \times 2{\cdot}5) = 270.$$

This method will allow fairly accurate comparisons to be made between infestations on different varieties of potatoes, on potatoes at different centres or grown under different conditions. So far, however, experiments have failed to show any close relationship between number of aphids, either caught on traps or counted on potatoes, and the spread of viruses (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948). Whitehead (1943), after comparing the number of aphids at different centres in North Wales with the markedly different rates of degeneration, remarked: 'The aphis count alone, therefore, does not necessarily measure the potential menace to the potato crop.' It seems likely that for survey purposes, an approximate estimate of the incidence of *M. persicae* is all that is required. Gorham (1935–45) described an extensive survey which has been carried on over a number of years in Canada. The nature of the infestation was noted as very light, light, medium or severe, and diagrams of infested leaves illustrating the four grades were distributed to the observers. But a very light infestation in Canada is apparently anything up to ten aphids per leaf, which would be considered a severe infestation in Britain.

It seemed possible that with the lower population usually present in Britain, especially in seed-producing areas, some method of estimating the population without counting the aphids might be found. The percentage of leaves infested appeared to bear some relation to the population at low levels of infestation; a suitable sampling unit for testing this would be a group of three leaves, one upper, one middle and one lower, per plant.

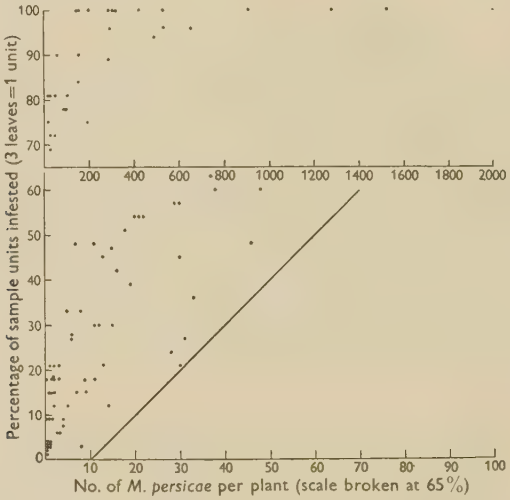

Fig. 8. The relation between percentage of sample units (groups of 3 leaves: upper, middle, lower) infested and number of *M. persicae* per plant, based on 33 sample units per field in 17 fields; 94 sampling occasions.

In Fig. 8 the percentages of such groups infested with *M. persicae* in fields sampled in 1946 and 1947 are plotted against the estimated number of *M. persicae* per plant on each occasion. The abscissa scale is changed at 65% so that the points against lower percentages can be clearly shown. A line can be drawn in the lower part of the figure delimiting the area occupied by the points, so that if 10% of the sampling units were infested, the population was below 20 per plant; if 60% of the units were infested the population was below 70 per plant; i.e. for infestation up to 60% the maximum population per plant expected may be obtained by adding 10 to the percentage figure. In the upper part of Fig. 8 a maximum population of 200 per plant was obtained at the infestation level of 70%, 400 at 80%, 700 at 90%, while at 100% the population varied from 150 to 2000 per plant. By this method the approximate population per plant (for low populations) or the likely upper limit of the population can be calculated.

It is questionable whether or not counts of individual aphids on the potatoes are necessary at any time for survey purposes. When counts are made, time in the field is spent in different ways as the season progresses: in the early part of the season

a relatively large number of plants have to be examined to determine the date of the first infestation; as the population builds up, more time is spent in making accurate counts of the aphids. If the estimation method is used no time will be saved in the early period, but a considerable amount of laborious and time-consuming work will be avoided when the population has grown. If it is assumed that an infestation of (say) over fifty per plant is likely to be dangerous for potato seed production in Britain and, as seems to be the case, low populations present on potatoes in the early season are of great importance, then the percentage of infested leaves will give the information required with as great an accuracy as a count of aphids. The information gained by making counts, which usually have an error of more than 20%, does not appear to warrant the time spent. The information sought in virus disease studies is more likely to be obtained by repeated visits to the field at short intervals and not from accurate counts on a few dates.

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ON ESTIMATING THE POPULATION OF APHIDS IN A POTATO FIELD

By F. J. ANSCOMBE
Rothamsted Experimental Station

This appendix to the preceding paper by L. Broadbent deals with the statistical problem of estimating the population of aphids per plant in a field of growing potatoes. An unbiased method is described, in which a definite proportion of a number of plants is sampled, and also a more rapid method of stratified sampling. (An account of the statistical principles involved has been given by Yates (1946).) Sampling for purposes of inspection is then considered, using practices current in industrial statistics and described, for example, by Peach (1947). Finally, some counts on *Myzus persicae* are examined, to obtain information on the type of variation in aphid numbers to be expected.

1. An unbiased method

The obvious method of estimating the number of aphids per plant in a field is to sample a definite proportion (e.g. every tenth leaf) of a number of plants. The plants are chosen by a random or systematic procedure that does not permit of personal choice by the observer, and the sampling of the individual plants is done by some such rule as:

(1) the leaves are enumerated by counting the leaves on each stem from top to bottom, and taking the stems in turn;

(2) each time the number 10 (or whatever number is chosen) is reached, the leaf is examined for aphids, and the next leaf is counted as number 1;

(3) on the first plant sampled, the first leaf is given a random number from 1 to 10, and not necessarily 1, while on the other plants the enumeration proceeds from where it left off on the previous plant.

Suppose N plants are examined in this way. Let r be the number of leaves examined on any plant and y the total number of aphids found on these r leaves. Then $10\Sigma y/N$ is an unbiased estimate of the number of aphids per plant in the field (if every tenth leaf has been examined). Its variance is, near enough,

$$\frac{100}{N}\,\mathrm{var}\,(y).$$

An unbiased estimate of the mean number of aphids per leaf in the field is given by $\Sigma y/\Sigma r$.

2. The three-leaf method

The above sampling method is rather laborious. It involves, in effect, counting the total number of leaves on each plant, and examining a large proportion of top leaves that contain few aphids. An easier procedure is to classify the leaves on a plant in three categories, upper, middle and lower, observe a fixed number of

leaves of each type per plant, e.g. one of each, to be chosen 'at random', i.e. at the observer's discretion, and separately to estimate the mean number of upper, middle and lower leaves per plant. If there is a correlation between the number of aphids per leaf and the number of leaves on a plant the method will be biased, and in any case the selection of leaves 'at random' by the observer is likely to lead to bias (e.g. he may tend to select large leaves). However, the method may be considered quite accurate enough for practical purposes, as we are interested in rather large differences between populations.

Suppose x_1, x_2, x_3 are the numbers of aphids found on a selected upper, middle and lower leaf of a plant, and N plants are examined. Let $\bar{r}_1$, $\bar{r}_2$, $\bar{r}_3$ be estimates of the average number of upper, middle and lower leaves per plant. Then the average number of aphids per plant is estimated as

$$\frac{1}{N}\{\bar{r}_1\Sigma x_1+\bar{r}_2\Sigma x_2+\bar{r}_3\Sigma x_3\}.$$

It is easier to obtain an accurate estimate of the number of leaves per plant than of the number of aphids per leaf. If the variances and covariances of $\bar{r}_1$, $\bar{r}_2$ and $\bar{r}_3$ are small, and x_1, x_2 and x_3 are uncorrelated with one another and with the number of leaves per plant, the variance of the above-estimated population per plant is roughly

$$\frac{1}{N}\{(\bar{r}_1)^2 \text{ var } (x_1)+(\bar{r}_2)^2 \text{ var } (x_2)+(\bar{r}_3)^2 \text{ var } (x_3)\}.$$

We have supposed here that equal numbers of upper, middle and lower leaves are examined for aphids. It may be asked whether some different proportion would be more economical of effort. Counts made in 1947 suggest that the relative numbers of upper, middle and lower leaves (i.e. $\bar{r}_1$, $\bar{r}_2$, $\bar{r}_3$) may be expected to be in the ratio 5:3:2 roughly in mid-season. On the other hand, whereas the numbers of aphids per leaf on middle and lower leaves are usually about equal (or at least do not seem to differ consistently), few aphids are normally found on top leaves. For a given total number of leaves counted, the most accurate estimate of number of aphids per plant will be obtained if the number of leaves of any type examined (upper, middle or lower) is proportional to the number of leaves of that type per plant multiplied by the standard error of the number of aphids per leaf of that type. It is doubtful whether the simple rule of examining equal numbers of upper, middle and lower leaves can be much bettered.

3. Inspection methods

If the object of sampling a field is to decide whether or not the infestation is satisfactorily low for the production of seed potatoes, it is permissible to use a simple inspection procedure that will be considerably quicker than the previous method if the infestation is high. It is necessary, however, to know definitely (in advance) by what standard the field is to be judged, and then the observations can be directed

towards seeing whether the field meets the standard. There is no need to give an estimate of the actual population of aphids.*

Maldwyn Davies (1934), working in north Wales, suggested that an infestation of not more than twenty *Myzus persicae* per 100 lower leaves in mid-July was satisfactory, while more than one hundred per 100 leaves was serious. Let us consider an inspection scheme based on this recommendation. If on a plant we observe simply whether an upper, a middle and a lower leaf carry between them any *M. persicae*, then Maldwyn Davies's low level would mean that some 75% or more of the plants would be clear (i.e. the three leaves uninfested), while his high level would give under 40% of plants clear. (The basis of this calculation is explained in § 5 below.) If the inspector observes thirty plants in this way, and passes the field as satisfactory if fewer than fourteen plants are infested, but rejects it if he finds fourteen plants infested, then a field at Maldwyn Davies's low level would be almost certainly passed, and a field at the high level would be almost certainly rejected (chance roughly 98% in each case).

The essential feature of the inspection method is that a certain portion of each plant selected is examined for presence or absence of aphids, and the portion examined is such that if the infestation is satisfactorily low there is a fairly high chance that it will not be infested, while if the infestation is high there is a fairly high chance that it will be infested. The proportion of plants showing infestation is then a good criterion. Broadbent (1948) has pointed out that it is unsatisfactory to base a standard on number of aphids per leaf and that number per plant is preferable, and he has shown empirically in his Fig. 8 how the percentage of three-leaf units infested is related to the population of aphids per plant. Knowledge is scanty, however, on the relation between aphid infestation and virus spread, and he has not recommended standards of acceptance and rejection based on aphid numbers. No detailed inspection scheme can therefore be proposed.

It should be noted that when three leaves, an upper, a middle and a lower, are examined per plant, it is rather unsatisfactory to calculate a straightforward percentage of leaves infested, as the three types of leaf will be differently infested. If, for example, the upper leaves are almost free, however heavy the infestation on the middle and lower leaves, the calculated proportion of leaves infested will never much exceed 2/3. By noting instead whether the three leaves carry any aphids between them, we eliminate this sort of anomaly; a high infestation leads to almost 100% of three-leaf units being infested.

* The present Ministry of Agriculture and Fisheries scheme for the inspection of growing crops of seed potatoes does not include any such test of aphid numbers. The subject is mentioned here to draw attention to the way in which sampling effort can be cut when the object is to decide a clear inspection issue and not simply to estimate the intensity of infestation for research purposes.

4. Analysis of counts on every tenth leaf

Some counts of aphids on every tenth leaf were made by Broadbent on experimental potato plots at Rothamsted in 1946, but the infestation was so low that nothing useful emerged. Similar counts were made in 1947 on two occasions, 21 July and 6 August, the infestation this time being abnormally high. The counts were as complete as possible, including information on size and type of leaf, and in addition the same plants were sampled by the three-leaf method. The only remaining information available on sampling definite proportions of plants consists of three counts made in 1946, at Postland and Baston (Lincolnshire) on 29 July and Bretton (Derbyshire) on 30 July, when every fifth leaf on every third or fourth stem was sampled. Unfortunately, the size of the plants was not recorded, and as the data are not extensive they have proved of little help in this investigation.

In the Rothamsted tenth-leaf count on 21 July 1947, twenty-six plants were sampled. The number of leaves examined varied between 6 and 23, with mean 15·6 and standard deviation 4·5. The average number of *M. persicae* per plant was estimated at 536 ± 64. The corresponding estimate by the three-leaf method, using information derived from the tenth-leaf count on the average number of upper, middle and lower leaves per plant, was 567 ± 65.

In the Rothamsted tenth-leaf count on 6 August 1947, ten plants were sampled. The number of leaves examined varied between 7 and 35, mean 20·9, standard deviation 9·3. The estimated number of *M. persicae* per plant was 967 ± 240 by the tenth-leaf method and 1011 ± 194 by the three-leaf method. (These standard errors are themselves subject to high estimation errors.)

It is not possible to compare the efficiencies of the two methods reliably, as the tenth-leaf counts were done in greater detail than would ordinarily be necessary, and a separate estimate of the number of upper, middle and lower leaves per plant was not made with the three-leaf method. But it seems that the latter was somewhat more economical of effort, in proportion to accuracy achieved. Considering the counts on upper, middle and lower leaves separately, the tenth-leaf counts and the three-leaf counts on the same plants are in fair agreement as to the average infestation per leaf, but the three-leaf counts seem somewhat less variable. This explains the roughly equal standard errors for the two methods quoted above, when we should have expected the three-leaf method to give rather higher standard errors. The effect is presumably due to chance.

In a further analysis of the tenth-leaf counts, the infestation per leaf appeared to be independent of the size of plant. There was no definite evidence of a component of variance in aphid numbers between plants in addition to the variance from leaf to leaf within a plant (upper, middle and lower leaves being considered separately); the 1946 counts at Postland and Baston tended to confirm this. There was some evidence of correlation between the number of aphids on a leaf and the length of

leaf, but not such as to suggest that accuracy of estimation could be materially improved by taking leaf length into consideration.

5. Analysis of three-leaf counts

Three-leaf counts from some thirty fields (in most of which thirty-three plants were sampled), with infestations of *M. persicae* ranging from 0·3 to 3 aphids per three-leaf unit, have been examined. The distribution of aphid numbers per unit appears to follow the negative binomial form, such that if the mean number per unit is m the variance from unit to unit is

$$m+\frac{m^2}{k}$$

and the proportion of units uninfested is

$$\left(1+\frac{m}{k}\right)^{-k}.$$

k appears to have roughly the same value for all fields, namely, 0·7. More exactly, there was evidence that it varied a little, and in particular tended to be larger for large values of m, but it will be accurate enough to take $k=0{\cdot}7$ as typical. (With really high infestations k may well be considerably larger.)

A simple approximate expression for the accuracy of the three-leaf method can now be given. Let us suppose (what is not far from the truth with low infestations) that the upper leaves carry no aphids, and that the middle and lower leaves are equal both in number and infestation. We suppose that the infestations of the middle and lower leaves are independent, the counts being distributed in negative binomial distributions with $k=0{\cdot}35$. Then we find that if m is the average aphid count per unit the proportional standard error of the estimated mean number of aphids per plant is

$$\sqrt{\left[\frac{1}{N}\left(\frac{1}{0{\cdot}7}+\frac{1}{m}\right)\right]}.$$

Thus if $m=1$ and $N=40$, we find that the proportional standard error of the estimate of aphid population per plant obtained by the three-leaf method examining forty plants is $\sqrt{0{\cdot}0607}=0{\cdot}25$, i.e. the estimation error is $\pm 25\%$. Maldwyn Davies's low level of infestation gives $m=0{\cdot}4$, on the assumptions here made, and then the estimation error is $\pm 31\%$; while his high level gives $m=2{\cdot}0$ and an error of $\pm 22\%$.

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STUDIES ON *BACTERIUM FASCIANS**

V. FURTHER OBSERVATIONS ON THE PATHOLOGICAL AND PHYSIOLOGICAL REACTIONS OF *BACT. FASCIANS*

By MARGARET S. LACEY
Imperial College of Science and Technology, London, S.W. 7

(With 4 Text-figures)

Seedlings were treated with cell-free filtrates of cultures of *Bact. fascians*, *Bact. tumefaciens* and other bacterial species, and the effects on the growth noted and compared with malformations resulting from the inoculation of living bacteria. The power to produce or destroy growth-promoting substances in various culture fluids by *Bact. fascians* was examined and compared with that of other bacterial species. It is suggested that seedlings infected by *Bact. fascians* produce 'leafy-galls' or 'dwarfing' owing to a disturbance of the normal auxin content or distribution in the plant.

Previous papers (Lacey, 1936*a*, *b*, 1939, 1942) have described the isolation of *Bact. fascians* from abnormal plant growths and the successful inoculation of susceptible plants with this bacterium. The present paper describes further inoculation of seedlings with living *Bact. fascians* cultures and with bacteria-free culture extracts, and gives an account of the production and destruction of β-indole-acetic acid and other growth-promoting substances by *Bact. fascians* and other bacterial species.

Experimental

(1) *The growth of sweet-pea seedlings inoculated with* Bact. fascians

It has been shown (Lacey, 1936*a*, *b*) that inoculation of young sweet-pea seedlings with *Bact. fascians* caused the formation of basal 'leafy-galls' with stunting or, in severe cases, complete suppression of the primary shoot. Further observations on the disturbance of the general growth and metabolism of infected seedlings have now been made. In these experiments the sweet-pea seeds were sterilized with mercuric chloride, and after thorough washing with sterile water were placed in moist sterile sand in glass pots. In some cases the seeds were inoculated immediately after sterilization; in others, inoculation was deferred until germination had taken place. Under the conditions of these experiments the control, untreated, seedlings made good growth for 5–7 weeks, when the experiments were concluded.

The following observations are of interest:

(*a*) When ungerminated seeds were inoculated with *Bact. fascians* the percentage of germination was usually considerably higher than that of the sterile controls.

* Forming No. XXVI of 'Studies in Bacteriosis'.

(*b*) Slight stimulation of the rate of germination and of the initial growth of the plumule frequently occurred as the result of inoculation with *Bact. fascians*. After this initial stimulation growth of infected seedlings proceeded at a normal rate until 10–15 days after germination, when further increase in length of the primary shoot almost or entirely ceased. This cessation of growth usually coincided with the appearance of hypertrophied basal shoots, but occasionally the stunting of the primary shoot was the only sign of disease.

(*c*) In most cases the apical bud of *Bact. fascians*-infected seedlings was not diseased, but occasionally this showed marked fasciation and galls were also sometimes produced from buds in the leaf-axils of the primary shoot. Most of the abnormal growth, however, developed from the normally dormant buds at the base of the shoot.

(*d*) Occasionally, instead of stunted growth due to shortening of the stem internodes, the reverse occurred and shoots with abnormally long thin internodes developed. In both cases the leaves were markedly reduced in size, sometimes remaining rudimentary.

(*e*) In some experiments seeds were infected with a saprophytic bacterium (*Pseudomonas fluorescens* sp., a frequent contaminant on isolation plates prepared from plant tissues) either alone, or in combination with *Bact. fascians*. Occasionally, slight retardation of germination and initial plumule growth followed inoculation of seeds with *Ps. fluorescens* alone, but later growth was as good as, or better than, that of untreated seeds and no malformations occurred. When, however, seeds were inoculated with a mixture of *Bact. fascians* and *Ps. fluorescens* germination was markedly retarded, plumule growth was feeble or absent and the seedlings became more severely diseased than those inoculated with pure cultures of *Bact. fascians*, a wart-like growth of undifferentiated tissue frequently being the only development. In this connexion it is interesting to note that inoculation of impure cultures of *Bact. fascians* taken from the first isolation plates from plant galls were often decidedly more virulent than the final pure cultures. Fig. 1 illustrates these points. The plumules of seeds inoculated with *Bact. fascians* (Str. 12) emerged on the 5th day, and their growth was more rapid than those of the controls until the 8th day. Growth of basal shoots was visible in some of the infected seedlings on the 13th day, and simultaneously the growth rate of the primary shoots fell rapidly. The growth rate of seedlings infected with *Ps. fluorescens* closely paralleled that of the control seedlings in this experiment. The seeds infected with a mixed culture of *Bact. fascians* and *Ps. fluorescens* showed no sign of germination until the 11th day; some of the seeds produced 'warts' only and in others a short primary shoot developed in addition to the mass of basal gall tissue. After 6 weeks the average lengths of the primary shoots were: control and *Ps. fluorescens*-infected seedlings, 7·0 in.; *Bact. fascians*-infected seedlings, 3·0 in.; those infected with both *Bact. fascians* and *Ps. fluorescens*, 1·3 in. only.

In several experiments the seedlings were removed from the pots after 5–7 weeks'

growth, the roots and galls freed from sand and the appearance and weights of roots, shoots and galls compared. Except in very severely diseased cases, there was no significant difference between the total weights of control and *Bact. fascians*-infected seedlings, the gall tissue being produced at the expense of the stem and root. Although the roots of infected seedlings were reduced in size they were normal in appearance; no fasciation of root tissue was ever observed. The roots and

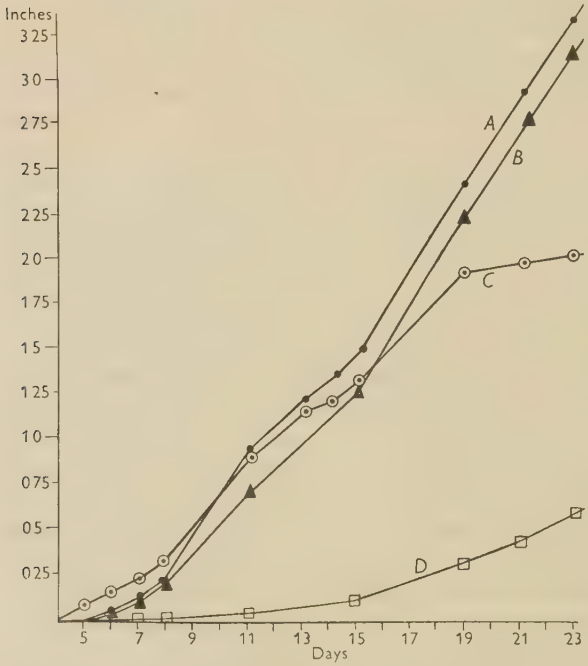

Fig. 1

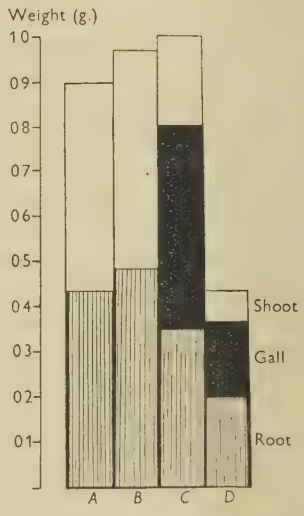

Fig. 2

Fig. 1. Growth of sweet-pea seedlings infected with *Bact. fascians*. *A* = control seedlings, *B* = *Ps. fluorescens*-infected seedlings, *C* = *Bact. fascians*-infected seedlings, *D* = *Bact. fascians* + *Ps. fluorescens*-infected seedlings.

Fig. 2. Comparison of average weights of root, shoot and gall tissue of sweet-pea seedlings. *A* = control seedlings, *B* = *Ps. fluorescens*-infected seedlings, *C* = *Bact. fascians*-infected seedlings, *D* = *Bact. fascians* + *Ps. fluorescens*-infected seedlings.

shoots and also the total weights of seedlings infected with a mixture of *Bact. fascians* and *Ps. fluorescens* were all greatly reduced. Fig. 2 compares the average weights of roots, shoots and gall tissue of seedlings in the same experiment as Fig. 1. The average weight of both roots and shoots of *Ps. fluorescens*-infected seedlings was slightly higher than that of the controls; *Bact. fascians*-infected seedlings showed a slight decrease in root and a considerable decrease in shoot weight, half the total weight being due to gall tissue. The average total weight of seedlings infected with

mixtures of *Bact. fascians* and *Ps. fluorescens* (namely, 0·42 g.) was less than half that of the other three series and was composed of 0·15 g. of gall tissue, 0·19 g. root and only 0·08 g. shoot.

(2) *Comparison of* Phaseolus vulgaris *seedlings infected with* Bact. fascians *or* Bact. tumefaciens

Young seedlings of *Phaseolus vulgaris* (with the first pair of leaves opened) were inoculated either with *Bact. fascians* (three strains) or with *Bact. tumefaciens* by smearing the apical buds and the leaf-axils with water suspensions of the organisms. One of the leaf-axils of each plant was then pricked with a needle. Plants infected in this way by *Bact. tumefaciens* produced typical galls at the site of injury only; the apical buds developed normally, and the inoculation had no effect on the general growth of the plants. The apical buds of plants inoculated with *Bact. fascians* sp. continued growth for a time but the internodes were short and swollen, causing stunting comparable to that of infected sweet-pea seedlings. In some cases growth in length ceased after two or three internodes had been produced, and a gall-like growth developed from the apical meristem. Similar masses of gall tissue were produced in both the wounded and uninjured axils of the primary leaves from the normally dormant lateral bud meristems.

(3) *Treatment of plants with bacteria-free culture extracts*

Strains of *Bact. fascians* were grown in broth or plant-extract media, and after varying periods of incubation the cultures were freed from bacteria by filtration through a Seitz filter. The filtrates were either used directly or concentrated and taken up in lanoline for application to stems of plants. Control plants were treated with uninoculated media. In some cases culture extracts of *Bact. tumefaciens* and other bacterial species were prepared in the same way and used for comparison with the *Bact. fascians* extracts.

(*a*) *Treatment of sweet-pea seeds with bacteria-free culture extracts*

Sweet-pea seeds were grown in sand to which equal quantities of culture extract, uninoculated media or water were added. Treatment with broth extracts was unsatisfactory, as both the culture extracts and the uninoculated media were injurious to the seedlings, the latter more so than the former. Better results were obtained with plant-extract media, but with these also the seedlings grown in water only were healthier than either of the treated lots. The experiments, however, gave some interesting results, the chief of which was stimulation of germination and early growth of the plumules in seeds treated with *Bact. fascians* culture extracts, similar to that obtained by the inoculation of living cultures. On the contrary, in two experiments in which *Bact. tumefaciens* culture extracts were used in comparison with *Bact. fascians* extracts, the former caused depression of the rate of germination and early plumule growth. For example, in one experiment, 5 days after the seeds

were treated, 44% of the *Bact. fascians* extract series had germinated and developed plumules up to 0·5 in. long, compared with 20% of the water series, 12% of the control medium and only 4% of the *Bact. tumefaciens* extract series.

In several of these experiments there were indications of stimulation of basal- or lateral-bud development in *Bact. fascians* extract treated seeds. This was most marked in an experiment in which sweet-pea seeds were treated with a culture filtrate of *Bact. fascians* grown in a synthetic medium with the addition of 0·05% yeast extract; a little fresh filtrate being added at weekly intervals to the sand in which the seedlings were grown. Only eleven of the sixteen seeds which were treated germinated and three of these died soon after germination. The remaining eight developed normally for 3 weeks, when two of the seedlings showed lateral bud growth. Six days later two more and finally another two also developed lateral growths. In two cases these side shoots were definitely fasciated, one being a wart-like growth, the second having short, swollen internodes. No lateral growths were developed in any of the controls.

(*b*) *Treatment of* Phaseolus vulgaris *with bacterial extracts*

In a number of experiments *Phaseolus vulgaris* seedlings were treated with lanoline paste containing culture extracts of *Bact. fascians*, *Bact. tumefaciens* or *Ps. fluorescens* sp. In some cases the paste was applied to the cut surfaces of decapitated stems or petioles, in others the paste was smeared on the apical buds, stems or petioles with or without preliminary scarification with a fine needle. The general response of seedlings treated with *Bact. fascians* extract paste was the shortening and thickening of treated internodes and petioles, but this also occurred with *Bact. tumefaciens* and *Ps. fluorescens* pastes. When apical buds were smeared with *Bact. fascians* paste the new growth was stunted (as in seedlings inoculated with living *Bact. fascians*), and slight fasciation of apical and lateral buds and epinasty of treated stems and petioles was frequently observed. Gall formation on injured surfaces was obtained with *Bact. tumefaciens* extracts (Brown & Gardner, 1936) and callus growth followed treatment with uninoculated media, but cut surfaces treated with *Bact. fascians* extracts produced neither gall nor callus.

The effect of *Bact. fascians* extracts varied considerably with the medium used and the age of the culture. For example, in one experiment, extracts of 5–12-day-old pea-medium cultures applied to the apical ends of bean seedlings caused marked inhibition of growth, but extracts of 3-week- to 2-month-old cultures caused stimulation of apical growth for the first week after treatment.

(*c*) *Treatment of lettuce seeds with culture extracts*

The culture media in most of these experiments were prepared from young lettuce seedlings grown in the dark. The shoots were ground up, mixed with water in the proportion of 1 ml. of water to each shoot, and after several hours standing the solution was filtered through paper and sterilized in flasks in the autoclave. After

inoculation with *Bact. fascians*, *Bact. tumefaciens*, etc., the solutions were inoculated for varying periods, usually 6 days, at 25° C. The bacteria were then removed by filtration through Seitz filters.

Lettuce seeds, sterilized in mercuric chloride removed by thorough washing in sterile water, were placed in small test-tubes and 1 ml. of the culture extract was added for each lettuce seed, 100 seeds being treated with each filtrate. Observation of germination and shoot and root growth was continued for about 14 days, after which the control seedlings grown in water ceased further growth. In the course of the experiment culture filtrates of several different strains of *Bact. fascians* and also of *Bact. tumefaciens*, *Ps. fluorescens* sp., *Bact. carotovorus*, *E. coli* and *Bact. aerogenes* were used. The following results were obtained:

(1) Lettuce seeds germinated and grew well in tap water for 12–14 days, producing normal roots and shoots which reached a length of about 3 cm. during this period.

(2) Seedlings grown in *Bact. tumefaciens*, *Ps. fluorescens*, *Bact. carotovorus*, *E. coli* or *Bact. aerogenes* culture filtrates developed very short, swollen 'peg-top'-shaped roots, which reached a maximum length of 0·5 cm. and were covered with a mat of root hairs greatly in excess of normal growth. A marked ring of swollen tissue commonly developed in the hypocotyl region.

(3) Uninoculated media caused similar but less marked root hypertrophy, the average length of the roots being 1 cm.

(4) Roots of seedlings grown in *Bact. fascians* culture extracts, though usually somewhat shorter than those of the water controls, developed normally.

(5) Germination and early growth of the shoot of *Bact. fascians*-treated seeds was usually slightly delayed compared with the water controls. This growth lag was greater in the seeds treated with uninoculated medium and very marked in those treated with all the other bacterial filtrates. Later development of the shoot was normal in every case.

Various tests were made on the bacterial culture filtrates and on the uninoculated medium to discover the reason for the marked root abnormalities, and it was found that, whereas the *Bact. fascians* extracts gave negative auxin tests, uninoculated lettuce-extract media gave a positive test, and the auxin content of *Bact. tumefaciens*, *Ps. fluorescens*, *Bact. carotovorus*, *E. coli*, and *Bact. aerogenes* culture fluids was much stronger than that of the uninoculated medium.

In order to find out whether the root abnormalities of treated lettuce seedlings could be correlated with growth substances present in the filtrates, lettuce seeds were grown in a series of dilutions of β-indole-acetic acid. It was found that a dilution of one part of β-indole-acetic acid in one to five million parts of water produced effects on the roots identical with those of the *Bact. tumefaciens*, etc., filtrates, while stronger solutions produced still more marked hypertrophy. The roots of seedlings grown in a 1/10,000,000 dilution resembled those grown in uninoculated media, while those in 1/100,000,000 β-indole-acetic acid were similar

to those of *Bact. fascians*-treated roots—shorter than those grown in water but of normal appearance and development (Fig. 3).

(4) *The production and destruction of growth-promoting substances in bacterial culture fluids*

The experiments on lettuce seedlings described above showed that *Bact. fascians* sp. differed markedly from the other bacterial species tested in behaviour towards growth-promoting substances in culture fluids. Earlier experiments with bacto-tryptone broth cultures had shown that, whereas 1–3-week-old cultures of *Bact. tumefaciens*, *E. coli*, *Bact. carotovorus* and a number of other bacterial species contained a considerable amount of β-indole-acetic acid, cultures of *Bact. fascians*

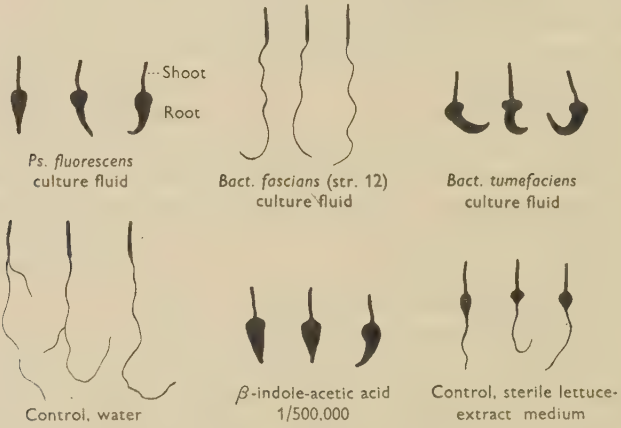

Fig. 3. Roots of lettuce seedlings grown in bacterial culture filtrates.

gave a negative colour test for this substance. Also, when a known amount of β-indole-acetic acid was added to a peptone-broth medium before inoculation, culture fluids of four strains of *Bact. fascians* gave completely negative colour tests for this substance after 3 days' incubation; the other *Bact. fascians* strains exhibited varying powers of destruction of β-indole-acetic acid, a few being quite inactive. On the contrary, culture fluids of all the other bacterial species tested—*E. coli*, *Bact. tumefaciens*, etc.—showed a definite increase in the concentration of β-indole-acetic acid in the same period.

Further experiments were then made to test the action of *Bact. fascians* on growth-promoting substances present in plant-extract culture fluids. A modification of Went's pea-seedling curvature test for auxin was employed in these tests, lettuce seedlings, which gave stem curvature similar to those of peas, being used instead of the latter. The advantage of using lettuce seedlings was the rapidity and ease of growth (the seedlings were ready for use in 4–7 days and were much less liable to infection by moulds than peas grown under similar conditions), and the small quantity of test fluid required (1 ml. of liquid for each lettuce shoot gave satisfactory

results). To obtain a standard of sensitivity of reaction of the lettuce seedlings, tests were made with β-indole-acetic acid and β-indole-butyric acid, both of which caused positive curvatures in dilutions of 1/20,000,000.

It was found that a medium consisting of lettuce shoots, grown in the dark and ground up with the addition of 1 ml. of water to each shoot and sterilized by autoclaving, usually gave a feebly positive auxin test. When such a medium was inoculated with *Bact. fascians* cultures the four strains of *Bact. fascians* (str. 12, 405, 421 and 448) which destroyed β-indole-acetic acid in broth culture also caused the destruction or inhibition of the auxin present in the lettuce medium. On the contrary, *Bact. tumefaciens*, *Ps. fluorescens*, *Bact. carotovorus*, *E. coli* and other bacterial species increased the auxin content of the fluid and also produced growth-promoting substances in those plant-extract fluids which gave negative auxin tests before inoculation (Fig. 4).

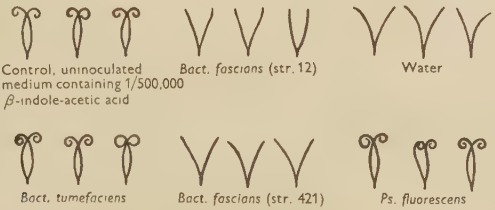

Fig. 4. Lettuce-seedling curvature tests of 3-day-old bacterial cultures in medium containing 1/500,000 β-indole-acetic acid.

In one experiment a 3-day-old culture of *Bact. tumefaciens*, giving a very strong positive auxin test, was sterilized by passage through a Seitz filter and tubes of the filtrate were inoculated either with str. 12 (an auxin-destroying strain of *Bact. fascians*) or *Ps. fluorescens*. After 3 days' growth the *Bact. fascians* culture fluid gave a negative auxin test, while the uninoculated filtrate and the *Ps. fluorescens* culture fluid gave the same strong positive reaction as the original *Bact. tumefaciens* culture. Also when *Bact. fascians* (str. 12) was grown in peptone broth in combination with *E. coli* or *Bact. carotovorus* (both faster and more vigorous growers than *Bact. fascians*) no β-indole-acetic acid was detected in the culture fluids, although the control tubes containing pure cultures of the latter two species gave strong positive colour tests for this substance. *Bact. fascians* is thus capable of destroying growth-promoting substances produced by other bacterial species as well as that present in various media.

As stated above only four of the *Bact. fascians* strains showed marked anti-auxin and hetero-auxin activity. In order to determine whether there was any variation in individual colonies of a strain, cultures of three of the active and four of the inactive strains were plated out. With the exception of one of the inactive strains (444, which produced only smooth colonies), all produced two distinct types of 'rough' and 'smooth' colonies. It was found that both types of colonies from the three active

strains were active, although the 'smooth' type were somewhat stronger than the 'rough', but none of the colonies, whether 'rough' or 'smooth', from the inactive strains showed any activity. Colonies of both types from 'active' and 'inactive' strains were then tested for virulence to sweet-pea seedlings, but no difference in pathogenicity between 'rough' or 'smooth' colonies or between 'active' or 'inactive' strains could be detected.

On reisolation from sweet peas infected with 'rough' and 'smooth' colonies of an inactive strain (484, from 'cauliflower' of strawberry), all colonies from the 'smooth' type infection were 'smooth' and all from the 'rough' type were 'rough'. When two of each of these reisolated colonies were tested for anti-auxin activity by inoculation into broth containing β-indole-acetic acid all four cultures gave a negative colour test for this substance after three days' incubation, indicating an activity as strong as that of the four most active *Bact. fascians* strains. This test was repeated several times with the four reisolated colonies and with the original 'rough' and 'smooth' cultures, each time with the same result, i.e. the original cultures were completely inactive but the four reisolated colonies were still fully active when the last test was made three months after reisolation. All the cultures used in this work had been kept on artificial media for a considerable time and it seems possible that cultures of *Bact. fascians* when first isolated from plant growths would usually exhibit anti-auxin activity which in some cases is lost by cultivation on artificial media.

DISCUSSION

A serious disease known as 'dwarfing' affects many plant genera such as cauliflower, lettuce, polyanthus, primula and violets. In this disease the whole plant is reduced to a rosette bearing minute leaves, in which, in severe cases, the lamina may be entirely absent, the mid-rib alone being developed. On numerous occasions *Bact. fascians* sp. have been isolated from these 'dwarfed' plants but the cultures, although morphologically and physiologically similar to those isolated from leafy-galls, were almost or entirely avirulent to sweet-pea seedlings. It has been stated that in some cases the only abnormality exhibited by sweet-pea seedlings artificially infected by *Bact. fascians* was the stunting of the primary shoot with reduction in the size of the leaves. This effect was sometimes obtained as the result of inoculating sweet-pea seedlings with the 'dwarfed' strains of *Bact. fascians* but typical gall formation was absent.

In the last decade a considerable amount of information has been obtained on the role of natural plant auxins in seedling growth and on the effect of treating plants with growth-promoting substances such as β-indole-acetic acid, the latter mainly in comparison with abnormalities induced by *Bact. tumefaciens* cultures or culture extracts. Went (1938) says: 'One of the most startling discoveries in the field of plant growth hormones is that auxin affects not only the growth in length of stems, petioles, etc. but also inhibits lateral buds and induces root formation, swellings, cambial activity, etc.' He showed that to produce growth in length of stems or

lateral buds a factor coming from the roots (caulocaline) is required in addition to auxin. It is generally accepted that there is correlation between the auxin content of the primary stem and the degree of bud inhibition. When the primary stem is decapitated the auxin ratio of stem and buds is upset and the axillary buds develop.

Since Brown & Gardner (1936) first showed that growth-promoting substances such as β-indole-acetic acid produced galls on wounded plant surfaces similar to those induced by *Bact. tumefaciens* cultures or culture extracts (which contain β-indole-acetic acid) detailed studies by these and other workers have been made on the histological responses of various plants to these substances. It has been shown that both *Bact. tumefaciens* and β-indole-acetic acid, when applied to the cut surface of a decapitated stem, not only induce gall formation but also inhibit the growth of lateral buds.

Bact. fascians has the reverse effect. A virulent culture of this organism stimulates the growth of lateral buds to such an extent that 'leafy-galls' are produced and growth of the primary stem is stopped, while a feebly virulent strain induces 'dwarfing' only; *Bact. tumefaciens* does not affect normal stem growth. Also, *Bact. tumefaciens* can only induce galls on a wounded surface but *Bact. fascians* does not require the presence of wounds and culture extracts inhibit normal callus formation on an injured surface. It is generally thought that one factor in gall formation induced by *Bact. tumefaciens* is an excess of growth-promoting substances produced either by the plant or by the bacterium, which is known to produce β-indole-acetic acid in culture fluids. On the contrary, the experiments described in this paper show that *Bact. fascians* is not only unable to produce β-indole-acetic acid in culture fluids but that some strains are able to destroy this substance and also naturally occurring auxins present in plant-extract media. It seems possible that 'leafy-galls' or 'dwarfing' are the result of the loss or disturbance of the normal auxin content or of factors such as 'caulocaline' in seedlings infected with *Bact. fascians*.

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VARIETAL RESISTANCE OF FLAX TO DISEASE

By JOHN COLHOUN
Plant Disease Division, Ministry of Agriculture, the Queen's University of Belfast

Field experiments, using contaminated seed, have been carried out from 1941 to 1947 to study the reaction of a wide range of both fibre and oil varieties of *Linum usitatissimum* to attack by *Polyspora Lini* Laff., *Colletotrichum linicola* Pethybr. & Laff., *Phoma* sp. and *Melampsora Lini* (Ehrenb.) Lév. The tests made with *Colletotrichum linicola* were supplemented by direct inoculation experiments with young seedlings grown under greenhouse or laboratory conditions.

None of the varieties tested proved to be immune to attack by *Polyspora Lini*, *Colletotrichum linicola* or *Phoma* sp. Herkules, Concurrent, Hindi, La Plata, Saginaw × Ottawa 770 B and Winona × Ottawa 770 B were resistant to the stem-break and browning phases of attack by *Polyspora Lini*; Bison, Hindi and La Plata were resistant to attacks by *Colletotrichum linicola*, while Hindi and La Plata showed some resistance to *Phoma* sp. Immunity or a high measure of resistance to attack by *Melampsora Lini* was exhibited by Formosa, Stakhanovets, Textilshchik, U.S.S.R. No. 2 (ex Dugejheshchya), Wada, Argentine Selection (C.I. 112), Baladi, Bolley Golden (C.I. 644), Bombay (C.I. 42), Hindi, Italia Roma (C.I. 1005–2), J.W.S. (C.I. 708–1), Koto (C.I. 872), Kenya (C.I. 709–1), La Plata, Newland (C.I. 188), Ottawa 770 B (C.I. 355), Pale Blue Crimped (C.I. 647), Tammes Pale Blue (C.I. 333–1), Williston Golden (C.I. 25–1) and C.I. 836.

All varieties did not show the same reactions to stem-break as to the browning phase of attack by *Polyspora Lini* and similar differences were noted in the case of the uredospore and teleutospore stages of *Melampsora Lini*. In certain varieties the stems showed a greater resistance to attack by *Polyspora Lini* or *Melampsora Lini* than the leaves.

Seeds of all varieties tested in the field experiments became heavily attacked with *Polyspora Lini* (except in La Plata), *Colletotrichum linicola* and *Phoma* sp.

Introduction

Although most seed-borne diseases of flax are widely distributed comparatively little information on their severity on different varieties is available. On the other hand, much work has been done, particularly in America, on susceptibility to rust (*Melampsora Lini* (Ehrenb.) Lév.) and wilt (*Fusarium Lini* Bolley), and on the production of immune or resistant varieties.

Since this investigation was commenced in 1941 experiments have been made annually to study the reaction to disease of different varieties of flax in the field, the work being carried out at the Agricultural Research Institute, Hillsborough, Co. Down. Where necessary the field experiments have been supported by studies made under laboratory or greenhouse conditions. Special attention has been given to the three seed-borne parasitic fungi *Polyspora Lini* Laff., *Colletotrichum linicola* Pethybr. & Laff. and *Phoma* sp., but *Melampsora Lini* has also been included in

the scope of the investigation. During the work a considerable number of fibre-producing varieties and a selection of the oil varieties have been tested.

Historical review

Stem-break and browning (Polyspora Lini)

Henry (1934) proved by artificial inoculation that some oil varieties are more susceptible to this disease than others. He found that the variety Bombay and certain other Indian selections were among the most susceptible tested, whereas Winona and Ottawa 770B showed considerable resistance. Henry also pointed out that the same variety may show different reactions to different strains of the parasite. Flor (1936), as a result of both greenhouse and field experiments, concluded that Bison was the most susceptible of the commercial oil varieties he studied, with Redwing, Linota, Buda and Rio following in order of increasing resistance. In New Zealand, Baylis (1941) has studied the reaction of a number of fibre and oil varieties to *P. Lini* by spraying a suspension of spores from three isolates of the fungus over seeds which were then sown in randomized blocks. Although the plants were also sprayed with spore suspensions when they were 2–4 in. high and again when they were coming into flower, little stem-break developed but a severe attack of browning resulted. When the severity of browning on both leaves and stems was considered Baylis placed the varieties tested in the following order of increasing resistance: Stormont Cirrus, Stormont Gossamer, Liral Crown, Punjab, Newland, Redwing, Giza Purple, New Zealand Commercial, North Dakota 52, North Dakota 73, Moose 13/28, Concurrent, Bison, Buda, North Dakota 114, Maramko, Moose 11/29, Moose 16/28 and Rio. From these results he concluded that the highest degree of resistance was shown by the large-seeded oil varieties, Rio being very resistant, although microscopic examination showed that ripe sepals and fruits of this variety could be attacked. In Denmark, Johansen (1943) has studied the reaction of twenty flax varieties to *P. Lini*.

Seedling blight (Colletotrichum linicola)

Little appears to be known regarding the reaction of fibre varieties to this parasite. Oleynikova (1935) found in the U.S.S.R. that none of the varieties she tested was wholly resistant although Alpha and five other unnamed varieties showed a relatively low degree of susceptibility. Houston (1944) reported from California that Rio and two unnamed plant introductions possessed partial resistance. As a result of a comprehensive series of greenhouse experiments carried out in the U.S.A., Ray (1945) concluded that the fibre varieties tested showed little resistance to *C. linicola*, although this result is in disagreement with the findings of Hiura (1924) who found in Japan that the broad-leaved varieties of seed flax were very susceptible while the narrow-leaved varieties of fibre flax were comparatively resistant. Ray

also found that the large-seeded Argentine types together with Linota, Bison and Buda carried resistance.

Foot-rot (Phoma *sp.*)

Although foot-rot of flax caused by *Phoma* sp. is prevalent in most countries where the crop is grown, practically no information exists regarding the reaction of varieties to this parasite. The white-flowering varieties as represented by Texala, Bella and Excello were stated by De Jonge (1933) to be susceptible to *P. exigua* in Holland.

Rust (Melampsora Lini)

In most flax-growing countries, apart from the British Isles, observations have been made on the reaction of both fibre- and oil-producing varieties to rust and a substantial number of resistant varieties have been listed (Henry & Stakman, 1925; Henry, 1926; Vallega, 1938; Dillman, 1936; Cass Smith, 1942; Conners & Saville, 1943; Arny, 1943). When these investigations were being made it was found that a variety might give different reactions in different seasons in the same country, and also that the same variety might react differently in different countries. In order to explain such results it was suggested by Henry (1926) that biological strains of the fungus might exist, and this has been proved to be the case by the work of Flor (1935, 1940, 1946, 1947) supported by that of Straib (1939*a*, *b*, 1942) and Waterhouse & Watson (1941, 1944).

The existence of physiological strains was demonstrated by using young seedlings, and there is reason for believing that in the reaction of varieties towards rust certain varieties which show high susceptibility in the seedling stage may show a varying degree of attack in the later stages of development and more particularly so just at the time when damage to the fibre shows its full effect. Straib (1942) has shown that varieties which are susceptible to cotyledon and leaf attack may show only a low degree of infection on the stems during the whole period of growth and in some cases the stems may remain free from infection.

METHODS

Field experiments

In testing flax plants for their reaction to attack by *Polyspora Lini*, *Colletotrichum linicola* and *Phoma* sp., seeds of different varieties free from parasitic contamination were sown in drills each 12 ft. in length at a rate equivalent to 80 lb. per statute acre. Each of the drills alternated with a drill sown with seeds heavily contaminated with the parasite under examination, a distance of 1 ft. separating the drills. One particular sample of seed contaminated with the specific parasite was used in each year. All the varieties being tested for reaction to attack by a particular parasite were included in one block, one drill being sown with each variety. In certain years

the experiments were made in duplicate. Observations on the incidence of disease were made during the growing season and at pulling time, the method described by Muskett & Colhoun (1942) being followed. The seed crop from each drill sown with a variety under test was harvested separately and 500 seeds were examined by the Ulster method (Muskett & Malone, 1941) for contamination with seed-borne parasites. The seed harvested from each drill was sown in the following spring in an isolated 6 × 6 ft. plot for observation on the incidence of disease. This procedure was repeated for a third, and sometimes a fourth, year, but where a heavy attack of disease was experienced the amount of seed available even in the third year was very small. Sowing was always carried out by hand as described by Muskett & Colhoun (1942). In each year an attack by *Polyspora Lini* and *Phoma* sp. developed on the appropriate crops although the intensity of the attack varied from season to season. A satisfactory attack by *Colletotrichum linicola* did not always develop.

In 1941 plots each measuring 6 × 6 ft., and separated by paths 6 ft. in width were sown with seeds of a number of varieties, four plots being sown with seed of each variety. Before sowing the seeds were sprayed with a suspension of spores of *Polyspora Lini* or *Colletotrichum linicola*, and when the seedlings were 2–3 in. high two plots of each variety were sprayed with a suspension of spores of the fungus used for inoculating the seeds sown in those plots. Heavy attacks of the diseases did not result in most cases. The seed from these crops was harvested and treated in the same manner as that saved from plants grown in drills.

When testing the reaction of varieties to *Melampsora Lini*, seed of each variety was sown in a plot 6 × 6 ft. or in a drill 12 ft. in length with 1 ft. separating the drills. In most years one sowing was made at the normal time and a second was made about 1 month later. In each year a heavy attack of rust occurred especially on the late-sown crops. Observations were made at suitable times on the incidence of the uredospore and teleutospore stages following the same method as was employed for the other diseases.

Laboratory experiments

The reaction of varieties to *Colletotrichum linicola* has been studied when seedlings were sprayed with a spore suspension. The seedlings used for this purpose were grown either in pots or in small clumps in large boxes and were inoculated when the first foliage leaves were appearing. After inoculation the plants were kept in damp chambers for 4 days either in the greenhouse or in the open, depending on the temperature. The most satisfactory results were obtained when the temperature was in the region of 15–20° C. Observations on the reaction of each variety were made 3 or 4 days after the plants were removed from the damp chambers.

RESULTS

During the 7-year period of investigation a considerable amount of data was collected, but to save space the results obtained from representative experiments only are presented (Tables 1–4). The results obtained from all the experiments made have been summarized in Table 5, where the varieties have been classified according to their reaction to each parasite. In this classification the varieties have been divided into four groups—immune, resistant, susceptible and very susceptible.

TABLE 1. *Reaction of varieties of flax to* Polyspora Lini, 1944–6

	Crop examination. Maximum attack = 10.				
Variety	Browning 29. viii. 44	Stem-break 8. viii. 45	Browning 24. viii. 45	Stem-break 22. vii. 46	Browning 27. viii. 46
Fibre flax:					
Blenda	6	4	5	8	10
Blue Star	10	10	7	—	—
Concurrent	0	2	0	6	1
Dutch H	0	—	—	—	—
Giza Purple	6	2	1	5	10
Herkules	0	4	0	1	0
Hunter's Hybrid	3	6	10	8	1
J.W.S.	2	6	1	3	1
Linkopis	0	1	0	3	1
Liral Crown	6	4	7	3	3
Liral Dominion	10	8	10	8	6
Liral Duke	6	10	5	5	3
Liral Monarch	3	2	0	6	6
Liral Prince	6	6	10	8	6
M 11/21	6	4	2	3	6
Norfolk Earl	6	6	7	5	6
Norfolk Queen	2	6	2	3	3
Pinnacle	6	6	7	8	6
Stormont Cirrus	10	6	2	5	10
Stormont Gossamer	6	10	10	10	—
Stormont Motley	3	8	7	10	10
6/1 × Livonian II 2K3/1	6	8	7	5	6
6/1 × Livonian II 2D4/1	2	—	—	—	—
Dark-grain mutant from Liral Monarch	2	2	0	3	6
Oil flax:					
Baladi	10	6	10	—	—
Hindi	0	2	1	1	0
La Plata	6	1	2	1	0
Ottawa 829C	2	10	5	10	—
Redwing	3	2	2	5	3

	Date of sowing	Date of pulling
1944	3 May	29 Aug.
1945	24 April	24 Aug.
1946	25 April	27 Aug.

Stem-breaking and browning (see Tables 1 and 5)

Seedling attack. None of the thirty-three varieties tested showed marked resistance to the seedling phase of the disease. In some seasons slight differences in resistance were observed to exist between varieties, but they were not consistently maintained and the results are not given.

Stem-break. Among the fibre varieties the most resistant to the stem-break phase were Concurrent, Herkules and Linkopis, while a lesser degree of resistance was shown by Giza Purple, J.W.S., a dark grain mutant from Liral Monarch, M 11/21 and Norfolk Earl. Of the oil varieties tested La Plata exhibited the highest degree of resistance noted for any oil or fibre variety tested. Other oil varieties which exhibited some measure of resistance were Hindi, Saginaw × Ottawa 770 B and Winona × Ottawa 770 B, the two latter hybrids being tested in one year only.

Browning. The fibre varieties Concurrent and Herkules showed a considerable degree of resistance to the browning phase, Herkules being the more resistant. A lesser degree of resistance was shown by J.W.S., Linkopis and Norfolk Queen. Although the numbers of lesions appearing on the stems of these varieties were comparatively few the leaves were often severely affected. It should be noted that all the Liral and Stormont varieties are very susceptible. Of the oil varieties Hindi and Winona × Ottawa 770 B showed resistance of the same order as that exhibited by Herkules while La Plata, Redwing and Saginaw × Ottawa 770 B showed a lesser degree of resistance. The leaves of Redwing were very susceptible although the stems showed resistance. The varieties Winona × Ottawa 770 B and Saginaw × Ottawa 770 B were tested for one season only.

Contamination of seed. When the seeds harvested were tested for contamination with *Polyspora Lini* it was found that, with the exception of La Plata which was only tested for 3 years, a high percentage number of contaminated seeds were recorded for each variety although a substantial variation normally occurred from year to year. By sowing heavily contaminated seed it was possible to determine whether or not a variety possessed real resistance to crop attack, and in no case, except in that of La Plata, can the resistance attributed to a variety be regarded as being associated with the employment of seed samples which were only slightly contaminated. Thus in one year the samples of seed of the following resistant varieties used for sowing showed the stated percentage number of contaminated seeds: Herkules, 33·6, Concurrent, 33·4 and Linkopis, 26·4.

Seedling blight (see Tables 2 and 5)

In the field tests carried out from 1941 to 1943 it was found that none of the thirty-three varieties included showed marked resistance, and that seeds of all of them readily became contaminated with the parasite. Since *Colletotrichum linicola* causes the greatest damage in the seedling phase it was considered advisable to spray seedlings of the various varieties with a spore suspension and then to provide

TABLE 2. *Reaction of varieties of flax to* Colletotrichum linicola *in inoculation experiments*

(1 = resistant, 2 = susceptible, 3 = very susceptible.)

Group and variety	1944 (a)	1944 (b)	1945	1946	1947
Fibre flax:					
Beatall	—	—	—	3	3
Blenda	2	3	3	2	3
Blue Star	3	2	3	3	3
Concurrent	2	3	3	3	3
Danish 21	—	—	—	—	3
Danish 40	—	—	—	—	3
Dutch H	3	3	2	2	3
Dutch 3	—	—	—	3	—
Formosa	—	—	—	3	3
Giza Purple	2	3	3	3	3
Herkules	2	3	2	3	3
Hollandia	—	—	—	3	3
Hunter's Hybrid	2	3	2	3	3
J.W.S.	2	3	3	—	3
Linkopis	3	3	2	3	2
Liral Crown	3	3	3	2	3
Liral Dominion	2	3	2	3	3
Liral Duke	3	3	3	3	3
Liral Monarch	3	3	2	3	3
Liral Prince	2	3	3	3	3
Livonian II	—	—	—	3	3
M 11/21	2	3	2	3	3
Norfolk Earl	3	3	2	3	3
Norfolk Queen	3	3	1	3	3
Percello	—	—	—	2	—
Pinnacle	2	2	2	2	3
Pioneer	—	—	—	3	—
Rembrandt	—	—	—	3	3
Saginaw	—	—	—	—	3
Stakhanovets	2	3	2	3	3
Stormont Cirrus	3	2	3	3	3
Stormont Gossamer	3	2	3	3	3
Stormont Motley	3	3	3	3	3
Textilshchik	2	3	2	3	3
*U.S.S.R. No. 1 (ex Shakhunskaya)	2	3	2	3	2
*U.S.S.R. No. 2 (ex Dugejheshchya)	2	3	2	3	3
Wada	—	—	—	—	2
1288/12 (ex U.S.S.R.)	3	2	3	3	2
6/1 × Livonian II 2K3/1	—	—	3	3	3
6/1 × Livonian II 2D4/1	3	3	3	3	3
Dark grain mutant from Liral Monarch	2	3	3	3	3
Oil flax:					
Baladi	1	3	3	3	2
Bison	—	—	1	1	2
Hindi	1	—	2	1	1
La Plata	1	1	1	2	1
Ottawa 829C	1	1	3	3	—
Redwing	1	3	2	2	2

* Unnamed samples received from Russia ex Flax Seed Station stated.

conditions suitable for the development of an epidemic of the disease. Some forty-seven varieties were tested by this method from 1944 to 1947, at least one experiment being made in each year. The results obtained from the field and laboratory experiments are summarized in Table 5, from which it can be seen that only three varieties, La Plata, Hindi and Bison showed any substantial degree of resistance. In these three varieties the cotyledons were attacked, but the hypocotyls were not seriously affected and most of the seedlings survived. The following varieties have been classified as susceptible: Percello, Pinnacle, U.S.S.R. No. 1 (ex Shakhunskaya), Wada and Ottawa 829C; all the other varieties tested were very susceptible.

TABLE 3. *Reaction of varieties of flax to* Phoma *sp.*, 1945–6

Crop examination. Maximum attack = 10.

Variety	1945	1946
Fibre flax:		
Blenda	4	5
Blue Star	10	7
Concurrent	4	½
Dutch H	4	5
Giza Purple	2	2
Herkules	1	2
Hunter's Hybrid	8	10
J.W.S.	6	1
Linkopis	6	5
Liral Crown	4	7
Liral Dominion	6	2
Liral Duke	4	1
Liral Monarch	6	5
Liral Prince	6	7
M 11/21	6	1
Norfolk Earl	8	1
Norfolk Queen	6	2
Pinnacle	6	10
Stakhanovets	8	2
Stormont Cirrus	6	2
Stormont Gossamer	8	2
Stormont Motley	6	2
Textilshchik	2	2
*U.S.S.R. No. 1 (ex Shakhunskaya)	4	2
*U.S.S.R. No. 2 (ex Dugejheshchya)	6	1
1288/12 (ex U.S.S.R.)	6	7
Dark grain mutant from Liral Monarch	4	1
Oil flax:		
Baladi	10	—
Hindi	1	0
La Plata	1	1
Ottawa 829C	4	0
Redwing	8	5

	Date of sowing	Date of pulling
1945	24 April	24 Aug.
1946	1 May	9 Sept.

* Unnamed samples received from Russia ex Flax Seed Station stated.

Foot rot (see Tables 3 and 5)

Observations on the reactions of forty-three varieties were made during the years 1943–6. Each of the seven experiments carried out extended over a period of from 1–4 years, depending on when it was commenced.

Only two of the varieties tested, Hindi and La Plata, showed any marked degree of resistance. A much lower degree of resistance than that exhibited by these two oil varieties was shown by the following fibre varieties, all of which have been classed as susceptible: Concurrent, Giza Purple, Herkules, Liral Dominion, Liral Duke, Textilshchik, U.S.S.R. No. 1 (ex Shakhunskaya), U.S.S.R. No. 2 (ex Dugejheshchya) and Rembrandt. The latter variety was tested in one year only. The remaining varieties were very susceptible.

When examined after harvest it was found that the seeds of all the varieties employed had become very heavily contaminated with *Phoma* sp. The seeds of the resistant varieties used for sowing were heavily contaminated with the causal organism in each year after that in which the experiment was initiated. For example, in one year the seed samples of La Plata and Hindi used for sowing showed the percentage number of contaminated seeds to be 55·6 and 77·6 respectively.

Rust (see Tables 4 and 5)

TABLE 4. *Reaction of varieties of flax to* Melampsora Lini, 1947

Crop examination. Maximum attack = 10.

	Uredospore stage			Teleutospore stage	
Group and variety	9. viii. 47	19. viii. 47	28. viii. 47	12. ix. 47	7. x. 47
Fibre flax:					
Beatall	5	7	8	5	7
Blenda	7	7	7	5	10
Blue Star	5	10	10	10	10
Caxias	7	3	7	2	5
Concurrent	2	10	10	5	3
Curitiba	2	2	7	5	7
Danish 21	7	10	8	5	5
Danish 40	7	7	10	5	7
Dutch H		5	5	5	5
Dutch Purple	8	10	10	10	10
Dutch 3	7	7	10	10	10
Formosa	2	2	2	0	2
Giza Purple	7	5	7	2	7
Herkules	7	2	7	5	2
Hollandia	2	5	5	2	2
Hunter's Hybrid	7	10	7	8	10
J.W.S.	7	2	7	7	10
Linkopis	0	2	5	7	8
Liral Crown	7	5	7	7	7
Liral Dominion	0	2	5	5	3
Liral Duke	5	7	7	2	5
Liral Monarch	7	7	10	10	10
Liral Prince	7	10	10	10	10

TABLE 4 (*continued*)

Group and variety	Uredospore stage			Teleutospore stage	
	9. viii. 47	19. viii. 47	28. viii. 47	12. ix. 47	7. x. 47
Fibre flax:					
Livonian II	7	7	7	7	10
Lyngby No. 7	2	7	8	5	5
M 11/21	7	10	7	10	10
Norfolk Earl	7	10	10	10	7
Norfolk Queen	7	7	7	5	5
Percello	0	0	5	2	1
Pernau Crown 2	7	10	7	10	10
Pinnacle	2	5	7	5	7
Pioneer	7	5	10	7	8
Rembrandt	5	10	7	5	5
Saginaw	5	7	8	7	5
Stakhanovets	0	0	0	0	0
Stormont Cirrus	2	2	5	5	2
Stormont Gossamer	7	7	10	7	8
Stormont Motley	5	5	7	10	10
Sussex	2	2	7	5	7
Textilshchik	0	0	0	0	0
*U.S.S.R. No. 1 (ex Shakhunskaya)	5	7	7	5	7
*U.S.S.R. No. 2 (ex Dugejheshchya)	0	0	0	0	0
Wada	2	2	1	0	1
Winona	0	5	7	5	2
1288/12 (ex U.S.S.R.)	0	0	0	0	0
6/1 × Livonian II 2D4/1	5	10	8	5	8
6/1 × Livonian II 2K3/1	5	5	5	2	2
Dark grain mutant from Liral Monarch	5	5	10	10	10
Oil flax:					
Abyssinian (C.I. 701)	2	5	5	5	5
Akmolinsk (C.I. 515–1)	5	7	6	2	1
Argentine Selection (C.I. 112)	0	0	0	0	0
Baladi	0	0	0	0	0
Bison	7	7	8	5	3
Bolley Golden (C.I. 644)	0	0	0	0	0
Bombay (C.I. 42)	0	0	0	0	0
Buda (C.I. 270–1)	2	5	5	5	5
Hindi	0	0	0	0	0
Indian 13	2	2	2	8	8
Italia Roma (C.I. 1005–2)	0	0	0	0	0
J.W.S. (C.I. 708–1)	2	0	2	0	1
Koto (C.I. 872)	0	0	0	0	0
Kenya (C.I. 709–1)	0	1	2	0	0
La Plata	2	5	3	2	1
Newland (C.I. 188)	0	2	1	0	0
Ottawa 770B (C.I. 355)	0	0	0	0	0
Pale Blue Crimped (C.I. 647)	0	0	0	0	0
Redwing	2	5	2	2	1
Tammes Pale Blue (C.I. 333–1)	0	0	0	0	0
Willeston Brown (C.I. 803–1)	2	5	7	7	7
Willeston Golden (C.I. 25–1)	0	0	0	0	0
C.I. 836	0	2	0	0	0

Date of sowing 6 June 1947 Date of pulling 7 Oct. 1947

* Unnamed samples received from Russia ex Flax Seed Station stated.

TABLE 5. *Varietal reaction to disease. Summary of results* 1941–7

(The letters in the columns signify the following host reactions: I, immune; R, resistant; S, susceptible; and VS, very susceptible.)

Variety	*Polyspora Lini*		*Colleto-trichum linicola*	*Phoma* sp.	*Melampsora Lini* Rust	
	Stem-break	Browning	Seedling blight	Foot rot	Uredo-spore stage	Teleuto-spore stage
Fibre flax:						
Beatall	—	—	VS	VS	VS	VS
Blenda	S	S	VS	VS	VS	VS
Blue Star	VS	VS	VS	VS	VS	VS
Caxias	—	—	—	—	VS	S
Concurrent	R	R	VS	S	VS	VS
Curitiba	—	—	—	—	VS	VS
Danish 21	—	—	VS	—	VS	S
Danish 40	—	—	VS	—	VS	VS
Dutch H	VS	VS	VS	VS	VS	VS
Dutch Purple	—	—	—	—	VS	VS
Dutch 3	—	—	VS	—	VS	VS
Formosa	—	—	VS	S	R	R
Giza Purple	S	S	VS	S	VS	VS
Herkules	R	R	VS	S	VS	VS
Hollandia	—	—	VS	VS	S	S
Hunter's Hybrid	VS	S	VS	VS	VS	VS
J.W.S.	S	S	VS	S	VS	VS
Linkopis	R	S	VS	VS	VS	VS
Liral Crown	VS	VS	VS	VS	VS	VS
Liral Dominion	VS	VS	VS	S	VS	S
Liral Duke	VS	S	VS	S	VS	S
Liral Monarch	VS	VS	VS	VS	VS	VS
Liral Prince	VS	VS	VS	VS	VS	VS
Livonian II	—	—	VS	VS	VS	VS
Lyngby No. 7	—	—	—	—	VS	VS
M 11/21	S	S	VS	S	VS	VS
Norfolk Earl	S	S	VS	VS	VS	VS
Norfolk Queen	S	S	VS	VS	VS	S
Percello	—	—	S	S	S	R
Pernau Crown 2	—	—	—	—	VS	VS
Pinnacle	VS	VS	S	VS	VS	VS
Pioneer	—	—	VS	VS	VS	VS
Rembrandt	—	—	VS	S	VS	VS
Saginaw	—	—	—	—	VS	VS
Stakhanovets	—	—	VS	VS	R	R
Stormont Cirrus	VS	VS	VS	VS	VS	VS
Stormont Gossamer	VS	VS	VS	VS	VS	VS
Stormont Motley	VS	VS	VS	VS	VS	VS
Sussex	—	—	—	VS	VS	VS
Textilshchik	—	—	VS	S	R	I
*U.S.S.R. No. 1 (ex Shakhunskaya)	—	—	S	S	VS	VS
*U.S.S.R. No. 2 (ex Dugejheshchya)	—	—	VS	S	R	I
Wada	—	—	S	—	R	R
Winona	—	—	—	—	VS	S
1288/12 (ex U.S.S.R.)	—	—	VS	VS	S	S
6/1 × Livonian II 2D4/1	VS	S	VS	VS	VS	VS
6/1 × Livonian II 2K3/1	VS	S	VS	VS	S	R
Dark-grain mutant from Liral Monarch	S	S	VS	VS	VS	VS

* Unnamed samples received from Russia ex Flax Seed Station stated.

TABLE 5 (*continued*)

Variety	*Polyspora Lini*		*Colleto-trichum linicola*	*Phoma* sp.	*Melampsora Lini* Rust	
	Stem-break	Browning	Seedling blight	Foot rot	Uredo-spore stage	Teleuto-spore stage
Oil flax:						
Abyssinian (C.I. 701)	—	—	—	—	S	S
Akmolinsk (C.I. 515–1)	VS	S	—	—	VS	S
Argentine Selection (C.I. 112)	—	—	—	—	I	I
Baladi	VS	VS	VS	VS	R	I
Bison	VS	VS	R	VS	VS	VS
Bolley Golden (C.I. 644)	—	—	—	—	I	I
Bombay (C.I. 42)	—	—	—	—	I	I
Buda (C.I. 270–1)	—	—	—	—	S	S
Hindi	R	R	R	R	R	I
Indian 13	—	—	—	—	VS	VS
Italia Roma (C.I. 1005–1)	—	—	—	—	I	I
J.W.S. (C.I. 708–1)	—	—	—	—	R	R
Koto (C.I. 872)	—	—	—	—	I	I
Kenya (C.I. 709–1)	—	—	—	—	R	I
La Plata	R	R	R	R	R	R
Newland (C.I. 188)	—	—	—	—	R	I
Ottawa 770B (C.I. 355)	—	—	—	—	I	I
Ottawa 829C	VS	S	S	VS	—	VS
Pale Blue Crimped (C.I. 647)	—	—	—	—	I	I
Redwing	S	S	VS	VS	S	R
Saginaw × Ottawa 770B	R	R	—	—	—	—
Tammes Pale Blue (C.I. 333–1)	—	—	—	—	I	I
Williston Brown (C.I. 803–1)	—	—	—	—	VS	VS
Williston Golden (C.I. 25–1)	—	—	—	—	R	R
Winona × Ottawa 770B	R	R	—	—	—	—
C.I. 836	—	—	—	—	R	I

Observations were made in each of the years 1943–5 and 1947 during which seventy-two varieties were tested.

Uredospore stage. None of the fibre varieties was found to be immune, although the following showed a high measure of resistance which in most cases approximated to immunity for all practical purposes: Formosa, Stakhanovets, Textilshchik, U.S.S.R. No. 2 (ex Dugejheshchya) and Wada. The number of uredospore pustules observed on the test plants of most of these varieties was small. Among the oil varieties Argentine Selection, Bolley Golden, Bombay, Italia Roma, Koto, Ottawa 770B, Pale Blue Crimped and Tammes Pale Blue were immune while Baladi, Hindi, J.W.S. (C.I. 708–1), Kenya, La Plata, Newland, Williston Golden and C.I. 836 showed a high degree of resistance which in most cases could be regarded as approximating closely to immunity.

Teleutospore stage. Two fibre varieties, Textilshchik and U.S.S.R. No. 2 (ex Dugejheshchya), were found to be immune, while the other fibre varieties found to be resistant to the uredospore stage together with Percello and

6/1 × Livonian II 2K3/1 possessed a high degree of resistance to the teleutospore stage and can be regarded as almost immune. The following oil varieties showed immunity: Argentine Selection, Baladi, Bolley Golden, Bombay, Hindi, Italia Roma, Koto, Kenya, Newland, Ottawa 770B, Pale Blue Crimped, Tammes Pale Blue and C.I. 836, while J.W.S. (C.I. 708–1), La Plata, Redwing and Williston Golden showed resistance.

DISCUSSION

In this work an attempt has been made to determine the susceptibility of flax varieties to the diseases caused by *Polyspora Lini*, *Colletotrichum linicola* and *Phoma* sp. by sowing seed samples naturally and heavily contaminated with the parasite concerned. In procuring the initial samples of contaminated seed it was found that when the different varieties were grown in drills alternating with drills of diseased plants raised from contaminated seed, ample and equal opportunities were provided for the infection of plants of the varieties under test as the healthy and diseased plants came into close contact. This method of obtaining contaminated seed is regarded as being more satisfactory than that of the direct inoculation of growing plants with spores of the parasite.

It has already been shown that in the case of seedling blight and stem-break and browning the incidence of disease in crops of susceptible varieties is related to the percentage number of contaminated seeds among those sown, and that an epidemic of these diseases does not normally occur unless more than 5% of the seeds are contaminated (Colhoun, 1946). In the experiments described here all the seed samples used for sowing in any year after that in which an experiment was commenced showed the percentage number of contaminated seeds to be greatly in excess of 5% except in the varieties La Plata and 6/1 × Livonian II D4/1 used in tests with *Polyspora Lini*.

Melampsora Lini is not seed-borne in the same sense as *Polyspora Lini*, *Colletotrichum linicola* or *Phoma* sp.; it is carried on small pieces of flax detritus mixed with the seed. No effort was made in carrying out these experiments to ensure the presence of material contaminated with *Melampsora Lini* in the seed sample, but it was found, nevertheless, that an epidemic of rust developed in each season, the disease being more severe in late-sown crops.

It is very apparent from the results presented that none of the varieties commonly grown for fibre in the British Isles possesses marked powers of resistance to all four parasites. Fair powers of resistance to *Polyspora Lini* were shown by Herkules and Concurrent which were classed as susceptible to *Phoma* sp. and very susceptible to both *Colletotrichum linicola* and *Melampsora Lini*. No fibre variety was found which was classed as resistant to *Colletotrichum linicola* or *Phoma* sp., although Liral Duke and Liral Dominion were somewhat less susceptible to *Phoma* sp. as well as to the teleutospore stage of rust attack than the other well-known British fibre varieties. The following fibre varieties were very resistant to *Melampsora Lini*:

Formosa, Stakhanovets, Textilshchik, U.S.S.R. No. 2 (ex Dugejheshchya) and Wada.

The oil-flax varieties tested showed a much greater measure of disease resistance in general than the fibre varieties, Hindi and La Plata being found to be resistant to all four parasites. The hybrids Saginaw × Ottawa 770B and Winona × Ottawa 770B, which were tested for reaction to *Polyspora Lini* in one year but were not studied in relation to any other parasites, proved to be fairly resistant to both stem-break and browning, but further observations on their reaction are desirable. Bison proved resistant to *Colletotrichum linicola* but was very susceptible to attack by the other parasites. A very substantial number of varieties proved to be resistant or immune to *Melampsora Lini*, and a number of these are the strains employed by Flor (1935, 1940) for differentiating physiologic races.

The results show substantial agreement with those obtained by other workers. Baylis (1941) in tests with five fibre varieties found Concurrent to be resistant to *Polyspora Lini*, and he placed the oil variety Bison as being equal in resistance to Concurrent, but the results now presented show it to be very susceptible and agree with the finding of Flor (1936). The observation of Henry (1934) that Winona and Ottawa 770B possess considerable resistance to *Polyspora Lini* is of interest in view of the suggestion now made that the hybrids Saginaw × Ottawa 770B and Winona × Ottawa 770B show resistance. Ray (1945) reported Bison to be resistant to *Colletotrichum linicola*, and this variety, the only one used in these experiments which he found to be resistant, has again shown this character. The fibre varieties Stakhanovets, Textilshchik and U.S.S.R. No. 2 (ex Dugejheshchya), which have been developed in the U.S.S.R. for rust resistance, as well as the rust-resistant variety Wada raised in Australia (Millington, 1946), all proved to be resistant to the disease in Northern Ireland. Wada was bred from a few rust-resistant Riga Crown plants, and it is thought that the gene or genes concerned may be the same as those of the Russian resistant varieties (Thomas & Millington, 1946). Some differences occur between the reactions to *Melampsora Lini* of both the fibre and oil varieties used in this work and those obtained by some other workers, but these may be explained by the existence of physiologic races of the parasite.

During the course of this investigation it became apparent that a variety might show a different reaction to stem-break than to browning or to the uredospore than the teleutospore stage of *M. Lini*. Linkopis, for example, exhibits a higher measure of resistance to stem-break than to browning, while uredospore pustules of *M. Lini* were found on varieties such as Textilshchik, U.S.S.R. No. 2 (ex Dugejheshchya), Baladi, Hindi and Kenya which were always free from the teleutospore stage. In the case of *M. Lini* the differences observed may be due to the stems showing a greater measure of resistance than the leaves, an observation which has already been made for the varieties Redwing, Herkules and Concurrent in relation to their attack by *Polyspora Lini*.

Ray (1945) has discussed the relation between observations made in the field and

the greenhouse and concluded that the reaction to *Colletotrichum linicola* in the greenhouse is not inconsistent with the few field observations available. In the present investigation varieties which were susceptible in the field have been found susceptible in the greenhouse, but unfortunately the varieties which proved resistant under greenhouse conditions were not tested in the field.

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OBSERVATIONS ON A VIRUS DISEASE OF CERTAIN CRUCIFERS IN TRINIDAD

By W. T. DALE
Department of Mycology, Imperial College of Tropical Agriculture, Trinidad

(With Plate 7)

A virus disease of *Brassica juncea* Coss., *B. chinensis* L. and *Raphanus sativus* L. var. *hortensis* Backer occurs in Trinidad, but is not serious. Rape (*Brassica napus* L.), turnip (*B. rapa* L.) and *Lepidium virginicum* L. are susceptible, but cabbage, cauliflower, kohlrabi and the European radish seem to be immune. *Zinnia elegans* Jacq. and tobacco (*Nicotiana tabacum* L.) are also susceptible, but no infections were obtained on six other *Nicotiana* species, beetroot, Swiss chard and cowpea. Tobacco gives only necrotic local lesions, whereas the other hosts give no local lesions, but a systemic mottling, usually accompanied in crucifers by leaf deformation and stunting of the plant.

The virus is transmitted by *Rhopalosiphum pseudobrassicae* (Davis), which is probably responsible for spread in the field. Its thermal inactivation-point is 58° C., dilution end-point 1 : 1000, and longevity *in vitro* 4 days at 16–18° C. and 3 days at 24–30° C. The properties and host range of this virus are compared with those of others affecting the Cruciferae. It has affinities with some of them, but is considered to be distinct.

Introduction

A mosaic disease sometimes affects three cruciferous crops in Trinidad; namely, Indian mustard (*Brassica juncea* Coss.), pak-choi (*Brassica chinensis* L.) and an eastern variety of radish, known locally as moorei, which is presumably *Raphanus sativus* L. var. *hortensis* Backer. This is a larger plant than the European radish and has a white root which may exceed a foot in length. The incidence of the mosaic in these crops has so far been low and the resulting losses slight. The disease was transmitted from infected to healthy plants by mechanical inoculation and by the aphis *Rhopalosiphum pseudobrassicae* (Davis). Cross-inoculation experiments proved that the same virus is responsible for the disease in all three crops, on which the symptoms are essentially the same.

A number of plants, most of them belonging to the Cruciferae and Solanaceae, were tested for susceptibility to this virus. In mechanical inoculation experiments the juice of diseased plants was rubbed lightly over the leaves of test plants. Fine carborundum powder, sprinkled on the leaves of the latter prior to inoculation, increased the percentage transmission to susceptible hosts. All the susceptible species developed systemic symptoms, except tobacco, which was the only host to produce local lesions. The virus was transmitted to young mustard plants, apparently unchanged, from all hosts that became systemically infected, but it was not recovered from the local lesions on tobacco. Crucifers which were not infected by mechanical

inoculation also resisted infection by aphides. It proved impossible to recover the virus, by reinoculation to mustard, from plants on which no visible symptoms appeared, so these plants are immune and not tolerant. Susceptible hosts are listed below with brief descriptions of their symptoms.

Host range

Indian mustard (*Brassica juncea* Coss.) was readily infected experimentally, over 90% of the large number of plants mechanically inoculated developing symptoms in from 5 to 14 days, depending on their rate of growth. The characteristic light and dark green mottling and blistering of the leaves (Pl. 7, fig. 2) was sometimes preceded by an indistinct clearing of the veins; but this was frequently confined to a part of a leaf. Subsequently developing leaves were often pale green except for small irregular darker patches. Mustard plants infected at an early stage were severely stunted, particularly in the greenhouse, where the temperature sometimes reached 40° C. Pak-choi (*B. chinensis* L.) reacted to infection in a similar manner (Pl. 7, fig. 3), about 70% of mechanically inoculated plants becoming diseased. Three other varieties of Chinese cabbage were tested for susceptibility to the virus, namely, pe-tsai, chihli and wong-bok. Some writers refer these to *B. pe-tsai* Bailey, others to *B. pekinensis* (Lour.) Rupr. They were infected less readily than pak-choi, about 20% success being achieved. The incubation period of the disease in the four varieties approximated to that in mustard. Vein-clearing was the first sign of infection on moorei (*Raphanus sativus* var. *hortensis* Backer), this being followed by symptoms similar to those of infected mustard.

Turnip (*Brassica rapa* L.), var. Purple Top White Globe, also developed symptoms rather like those of mustard. About 60% of all inoculated plants became infected. The incubation period in turnip was longer than in mustard, symptoms appearing from 14 to 21 days after inoculation; but the test plants were growing rather slowly. Rape (*B. napus* L.), var. Dwarf Essex, was infected, both by inoculation and *Rhopalosiphum pseudobrassicae*. A rather indistinct vein-clearing, often confined to part of a leaf, appeared 8–10 days after inoculation, this being followed by an inconspicuous coarse mottling. *Lepidium virginicum* L., one of the few wild species of the Cruciferae found in Trinidad, is rare. It reacted to infection by a marked stunting of the whole plant. The leaves became reduced in size, distorted and mildly mottled. Symptoms appeared in about 14 days on 80% of the inoculated plants.

Zinnia elegans Jacq., the only species outside the Cruciferae on which systemic symptoms have so far been obtained, was infected with some difficulty. Vein-clearing was followed by a diffuse mottling, often confined to the base of the leaves. Symptoms were commonly absent from leaves developing some time after infection. Yellowish local lesions appeared on the inoculated leaves of White Burley tobacco (*Nicotiana tabacum* L.) 3 days after rubbing them with juice from mosaic-infected mustard plants. These lesions increased in size, sometimes up to 8 mm. in diameter,

a necrotic spot usually appearing in the centre (Pl. 7, fig. 4). Similar local lesions were produced on the leaves of an ornamental red-flowered tobacco variety, commonly referred to *N. tabacum* var. *macrophylla*. No systemic symptoms developed on either variety.

The following tested plants appear to be immune to the virus: *Brassica oleracea* var. *botrytis* L., cauliflower (Early Patna); var. *capitata* L., cabbage (Early Jersey Wakefield and Jersey Queen); var. *caulorapa* Pasq., kohlrabi (Early Purple Vienna); *Raphanus sativus* var. *radicula* (Pers.) A.DC., radish (French Breakfast and Burpee's Red Giant); *Beta vulgaris* L., beetroot; var. *cicla* L., Swiss chard (Lucullus); *Vigna unguiculata* (L.) Walp., cowpea; *Nicotiana glutinosa* L.; *N. rustica* L.; *N. sylvestris* Speg. & Comes; *N. sanderae* Hort.; *N. alata* var. *grandiflora* Comes; *N. chinensis* Fisch.

PHYSICAL PROPERTIES

The results of experiments to determine the thermal inactivation-point, dilution end-point and longevity *in vitro* of the crucifer virus are given in Table 1. Filtered

TABLE 1. *Inactivation temperature, tolerance to dilution and longevity* in vitro

Inactivation temperature, 10 min.

Temp. (° C.)	Plants inoculated	Plants infected	Temp. (° C.)	Plants inoculated	Plants infected
30	40	36	56	40	2
48	40	26	58	40	0
54	40	4			

Tolerance to dilution

Dilution	Plants inoculated	Plants infected	Dilution	Plants inoculated	Plants infected
0	40	38	1:1000	40	2
1:100	40	13	1:2000	40	0
1:500	40	5			

Longevity *in vitro*

16–18° C.			24–30° C.		
Age (days)	Plants inoculated	Plants infected	Age (days)	Plants inoculated	Plants infected
0	25	22	0	40	35
1	25	15	1	40	13
2	25	7	2	40	8
3	25	4	3	40	2
4	25	3	4	40	0
5	25	0	5	40	0
6	25	0			

juice from recently infected mustard was used, with healthy mustard seedlings as test plants. Although a host giving a local lesion reaction is usually best for this type of experiment, mustard was used in preference to tobacco because the latter behaves erratically and gives few lesions, even when inoculated with untreated juice. The thermal inactivation-point was found by heating samples of juice in thin-walled tubes for 10 min. in a water-bath at appropriate temperatures. Most

workers on crucifer viruses have stored samples of juice for longevity tests at or about 20° C. Unfortunately, this was not possible in Trinidad, so they were stored at 16–18° C. in one series of experiments and at laboratory temperatures varying between 24 and 30° C. in another. The dilution end-point was demonstrated in the usual manner, using filtered mustard juice. The thermal inactivation-point is the most reliable of the three physical properties for comparing viruses; that of the Trinidad crucifer virus lies between 56 and 58° C. Infections were obtained with juice diluted to 1 part in 1000, but not at 1 in 2000. The longevity *in vitro* is 4 days at 16–18° C. and 3 days at laboratory temperatures.

Transmission

Several hundred young mustard plants were raised from the seed of plants artificially infected with the virus at an early stage, but no evidence of seed transmission was obtained. Mechanical transmission cannot be important in the field, as the virus is not highly infectious and is therefore unlikely to be transmitted from diseased to healthy plants by cultural operations. Consequently, the incidence and spread of the disease must be governed by the prevalence and behaviour of insect vectors, of which the only one demonstrated so far is the aphis *Rhopalosiphum pseudobrassicae* (Davis). This insect was found to multiply rapidly on all the cruciferous crops grown in the greenhouse, proving very difficult to control and making the use of insect-proof cages essential. The aphis is commonly found on mustard, pak-choi and moorei in the field, particularly on mature seeding plants, which often harbour alate as well as apterous forms. It may also occur in the field on cabbage and cauliflower. The insect has been shown to be a reasonably efficient vector of the virus under suitable experimental conditions and probably accounts for most, if not all, of its spread in the field. The insect-virus relationship has not yet been studied in detail, but it is likely that the virus is of the non-persistent type (Watson & Roberts, 1939). In a preliminary test it was found that aphides became uninfective in less than 24 hr. after feeding on diseased plants; this lack of persistence of the virus in the vector contrasts with its longevity *in vitro* of 3 days at a corresponding temperature.

Identity of the virus

Mosaics affecting Chinese cabbage, turnip and related crops, described and figured by early workers, including Schultz (1921), Gardner & Kendrick (1921) and Clayton (1930), were apparently similar to the Trinidad disease, but few details were given. Table 2 gives a comparison of the host ranges and physical properties of those viruses of the Cruciferae which have been more comprehensively studied, data on the Trinidad disease being included. Pound & Walker (1945) considered that the more recently described crucifer viruses fall into two distinct groups, the majority being related to *turnip virus* 1 (Hoggan & Johnson, 1935) and the remainder to *cauliflower virus* 1 (Tompkins, 1937). The former are characterized by lower inactivation temperatures, 50–68° C. as against 75–80° C., and, in general, more marked symptoms

TABLE 2. *Comparison of properties and host ranges of certain crucifer viruses*

(After Table 2 of Pound & Walker, 1945.)

Virus	Authority	Physical properties: Inactivation temperature (° C. for 10 min.)	Physical properties: Tolerance to dilution	Physical properties: Longevity *in vitro* (hr. at 20° C.)	Host reactions: systemic symptoms (S); local lesions (L); no infection (o); not tested (–): Cabbage	Cauliflower	Kohlrabi	Rape	Turnip	Indian mustard	Chinese cabbage	Radish (European)	Tobacco	*Nicotiana glutinosa*	Swiss chard	*Zinnia elegans*
Cabbage ring necrosis	Larson & Walker (1941)	50	1:500	36	S	S	S	S	S	S	S	S	L	S	S	S
Cabbage black ring	Tompkins, Gardner & Thomas (1938)	59	1:10,000*	144*	S	S	S	S	S	S	S	S	L	S	o	–
Cabbage virus A	Walker, Le Beau & Pound (1945)	60	1:5,000	168	S	S	S	S	S	S	S	S	L	S	S	–
Turnip mosaic T8	Le Beau & Walker (1945)	58	1:2,000	168	S	S	S	S	S	S	S	–	L	S	S	S
Turnip virus 1	Hoggan & Johnson (1935)	54	1:10,000	48	S	–	–	–	S	–	–	–	L	S	–	–
Turnip mosaic	Chamberlain (1936, 1939)	60	1:100	48	S	S	–	S	S	–	–	–	L	–	–	–
Turnip mosaic	Tompkins (1938)	63	1:3,000	48	S	S	o	o	S	S	S	o	L	S	–	–
Turnip mosaic T9	Le Beau & Walker (1945)	58	1:10,000	120	o	o	S	S	S	S	S	–	L	S	S	S
Turnip mosaic T6	Le Beau & Walker (1945)	56	1:10,000	144	o	o	o	S	S	S	S	–	L	S	S	S
Turnip mosaic T1	Le Beau & Walker (1945)	56	1:10,000	216	o	o	o	S	S	S	S	–	o	S	S	S
Crucifer mosaic	This paper	58	1:1,000	96†	o	o	o	S	S	S	S	o	L	o	o	S
Rape mosaic	Ling & Yang (1940)	65	1:6,000	120‡	o	o	o	S	S	S	S	o	–	–	–	–
Mild stock mosaic	Tompkins (1939*a*)	60	1:4,000	120	o	o	o	–	S	S	S	S	L	L	–	–
Severe stock mosaic	Tompkins (1939*a*)	60	1:3,000	168	o	o	o	o	S	o	o	o	L	o	–	–
Radish mosaic	Tompkins (1939*b*)	68	1:14,000	336	S	S	S	o	S	S	S	S	S	L	–	–
Chinese cabbage mosaic	Tompkins & Thomas (1938)	75	1:5,000	72	S	S	–	S	S	o	S	S	L	L	–	–
Cabbage virus B	Walker, Le Beau & Pound (1945)	78	1:1,000	120	S	S	S	S	S	S	S	S	o	o	o	–
Cauliflower mosaic	Tompkins (1937)	78*	1:1,500*	336	S	S	S	S	S	S	S	S	o	o	o	–
Broccoli mosaic	Caldwell & Prentice (1942)	80	1:2,000	168	S	S	S	S	S	–	–	S	o	o	–	–

* As determined by Walker, Le Beau & Pound (1945). † At 16–18° C. ‡ At 11–13° C.
Brackets indicate immunological relationships (Pound & Walker, 1945).

at higher temperatures; the latter produce more severe symptoms at lower temperatures. While it has not been possible to study the effect of temperature on the expression of the Trinidad virus, it has been noticed that the disease is more severe on plants grown in the greenhouse, where the day temperature is consistently higher than in the field.

Little immunological and no serological evidence has yet been presented in support of this provisional grouping of crucifer viruses; nevertheless, the similarities between the properties and host ranges of some of them are too marked to be entirely fortuitous. The Trinidad virus shows a close resemblance to some of those ascribed to the *turnip virus* 1 group, but does not seem to be identical with any one of them. It is included in Table 2 in what seems to be a logical position with regard to its properties and host range. The last four viruses on the list are those which Pound & Walker (1945) ascribed to the *cauliflower virus* 1 group.

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EXPLANATION OF PLATE 7

Fig. 1. Healthy mustard leaf.
Fig. 2. Mustard leaf showing mosaic pattern and blistering caused by infection with the crucifer virus.
Fig. 3. Pak-choi leaf showing typical symptoms of virus infection.
Fig. 4. Tobacco leaf showing local lesions produced by the crucifer virus.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

STUDIES IN THE DIAGNOSIS OF MINERAL DEFICIENCY

IV. THE MINERAL CONTENT OF BARLEY PLANTS IN RELATION TO POTASSIUM DEFICIENCY

By D. W. GOODALL

*Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W. 7, and East Malling Research Station, East Malling, Kent.**

(With 4 Text-figures)

Material from four fertilizer trials on barley in Hampshire was analysed with the principal purpose of comparing the extent to which the potassium content of different plant organs was diagnostic of potassium deficiency. Samples of older leaf blades and sheaths, young leaves, stems and ears were gathered at the time of ear emergence from each of the 108 plots, and analysed spectrographically for calcium, iron, magnesium, manganese, potassium and sodium.

Differences in composition between plants from the different sites were proportionately greater in the older leaf blades than in the other organs for calcium, the young leaves for manganese and sodium, and the older leaf blades and sheaths for potassium. Differences in sodium and manganese content at the different sites appeared to be related to the differences in potassium status.

Applications of muriate of potash increased the potassium content of all organs except the ears, and decreased the content of magnesium, manganese and sodium, and of iron at one site. The effect of potassium supply on manganese and sodium content was most marked in the young leaves. The proportional increases observed in potassium content as a result of application of muriate of potash were similar at all four sites, in spite of the fact that responses in growth and yield differed greatly.

As between the four sites, the responses to muriate of potash observed in the yields of grain are significantly correlated with the potassium content of the older leaf blades and the stems, and the following tentative limiting values are put forward, above which no increase in grain yield as a result of potassic manuring may be expected:

(*a*) in the older leaf blades at the time of ear emergence 0·92 % potassium in dry matter,
(*b*) in the stems at the time of ear emergence 1·01 % potassium in dry matter.

Introduction

Prior to 1942, the author's investigations at East Malling on the diagnosis of mineral deficiency had been confined to the apple (Goodall, 1943, 1945, 1945*a*); but in view of the much greater wartime importance of field crops, particularly cereals, it was decided at the end of that year to concentrate attention on these crops rather than on fruit. The present paper is particularly concerned with potassium deficiency in barley.

The very extensive literature relating to the use of plant analysis for diagnostic purposes has been adequately reviewed elsewhere (Goodall & Gregory, 1947), so it will here be necessary only to refer to some of the attempts to use such methods for

* Present address: University of Melbourne, Australia.

the diagnosis of potassium deficiency in the small grains. The earliest investigator of this subject was probably Hellriegel (1867), who suggested, as a result of sand-culture experiments, that maximum yield of barley could be obtained only if the straw contained 0·5% K_2O, and the grain 0·38%, the potassium nutrition being inadequate if the analytical values were below these standards, and that plant analysis would give better indications of available nutrients than that of the soil. Wolff (1876, 1877) gave as the *absolut nothwendiges Minimum* for oats the value of 0·5% K_2O in the plant dry matter at the time of harvest, while 0·75% corresponded to good average development. Heinrich (1882) stated that the yield of oats could be expected to be low when the potassium content of the roots at maturity was no more than 0·1–0·2% K_2O. Atterberg (1888, 1901) found that the yield of oats suffered when the potassium content of the straw at maturity fell below 1·0% K_2O; as potassium became more deficient, the potassium content of the straw at maturity approached the value of 0·28% K_2O, while the corresponding value for the entire aerial part of the plant at flowering was 0·31%; the desirable level of potassium content, however, varied with the sodium supply. Helmkampf (1892) agreed broadly with Atterberg's conclusions, and regarded analysis of the tops at flowering time as the most promising diagnostic procedure. Liebscher *et al.* (1888) found that oats responded to potassic fertilizers when the potassium content of the straw at maturity was less than 1·0% K_2O. Godlewski (1901), using barley and wheat, and Stahl-Schroeder (1904), using oats, considered that the ratio of potassium in the plant to other nutrients provided a better index of the level of potassium nutrition than the potassium content itself, though in addition to his limiting ratios Godlewski was in agreement with Atterberg and Liebscher *et al.* (see above) in considering that a potassium content of the straw at maturity of less than 1·0% K_2O indicated an inadequate supply of the element. Hall (1905) likewise regarded analysis of the straw at maturity as a promising method of identifying potassium deficiency in barley. Wagner (1915), in the course of a report on an extensive series of fertilizer trials in Germany, stated that where rye had responded to potassic fertilizers the straw from the untreated plots always contained less than 1·15% K_2O at harvest, and usually less than 0·8%. For wheat, the potassium content of the straw at harvest below which response to potassic fertilizers always occurred was 0·67% K_2O, but no corresponding value was quoted for barley. Pfeiffer *et al.* (1915) differed from the other investigators mentioned in considering that plant analysis gave no useful indications of the potassium status of oats; Münter (1919) obtained more satisfactory results with wheat, but preferred to express his limiting values in the form of ratios of nutrients within the plant.

Among the most important investigations of more recent years in this field are those of Lundegårdh (1932, 1938, 1939, 1941, 1943). His data indicated that, for leaves of oats gathered between panicle emergence and flowering, a potassium content less than 20 m.equiv.% (40 when the phosphorus content was high) indicated the probability of a good response to muriate of potash. Leaves of plants actually

showing symptoms of potassium deficiency contained not more than 0·444% K. In Indiana (Thornton, 1932; Thornton *et al.* 1934) rapid tests were developed for the diagnosis of potassium deficiency in small grains, using an extract of the leaf bases. The extensive investigations at Montpellier (Maume & Dulac, 1934, 1934*a*, 1935, 1935*a*, 1936, 1936*a*, 1937, 1938, 1938*a*, 1942) must also be mentioned, in which the whole aerial parts of wheat plants were gathered at a closely defined stage of development—usually flowering—and the analyses were expressed in the form of triangular diagrams, with the nitrogen, phosphorus and potassium content data represented along the three sides of the triangle; these workers have not put forward any optimal or limiting values for which they claim general validity, but have confined themselves to comparative studies.

Experimental procedure

Among the fertilizer trials set up in 1943 by the Hampshire War Agricultural Executive Committee, in collaboration with the Advisory Chemist for the Southern Province, were four trials with barley at centres suspected of potassium deficiency. The trials were all in north-western Hampshire, at Chilbolton Down Farm, Stockbridge; Kimpton Down Farm, Andover; Leckford Estate, Stockbridge; and Snoddington Farm, Kimpton, Andover. The soils varied considerably in depth, but were all over a chalk subsoil. The experimental areas were situated within fields growing the same crop, managed commercially; they were designed as $3 \times 3 \times 3$ experiments, two of the degrees of freedom for the second-order interaction being confounded with differences among three blocks. The unit treatments were $\frac{3}{4}$ cwt. sulphate of ammonia, $1\frac{1}{2}$ cwt. superphosphate and $\frac{1}{2}$ cwt. muriate of potash per acre, the other plots receiving double these amounts, or none; each plot covered an area of $\frac{1}{60}$ acre. Significant responses in grain yield to muriate of potash were obtained at all sites but Leckford; to superphosphate at Chilbolton and Snoddington; and to sulphate of ammonia at Chilbolton only.

By the courtesy of those responsible for the trials, it was possible for the author to gather samples of barley and weeds for analysis from every plot. Only the barley samples will be considered in this paper. They were gathered on 14 and 15 June 1943, at which time the plants were at the stage of ear emergence, the ear either having just emerged, or being just about to emerge, from the last leaf sheath in the majority of stems on every plot. The sample taken from each plot consisted of twenty 1 in. lengths of drill-row, distributed at random over the plot, cut at half an inch above ground-level. They were transported to the laboratory and there, 1–3 days later, were separated into portions; the possibility must be admitted that some alteration in the distribution of mineral elements in the plants had taken place during this period. Each tiller in which an ear was developing was included in the analytical samples, and the portions separated were:

(*a*) the two oldest leaf blades still remaining green,

(*b*) the sheaths of these leaves,

(*c*) the whole of the youngest leaf,

(*d*) the whole stem, with leaf blades and sheaths removed,

(*e*) the ear.

The samples were prepared as described by Roach (1946), and dried in an electric oven at 80° C.

The samples were analysed spectrographically by a modification of the method described by Roach (1946). Modification was necessary, since the method as described led only to results based on subjective visual comparison of spectra, whereas for the present purpose objective results expressed numerically were required. Since no description of the modified method has been published, a brief account of the technique used will be included here.

In essence, Roach's method consists in the incorporation of 25 mg. of dry plant material in a spill of filter-paper, which is carbonized in ammonium chloride vapour and then fed mechanically into an oxy-acetylene flame. For purposes of photometric interpretation of the spectra, it was, however, necessary to incorporate in the optical system a step-sector rotating at 1400 r.p.m. in front of the spectrograph slit. This resulted in each spectrum photographed being divided into four portions, one representing the full exposure, the others $\frac{1}{2}$, $\frac{1}{36}$ and $\frac{1}{216}$ of full exposure respectively. This made it possible to obtain satisfactory readings from a single exposure for lines varying greatly in sensitivity. Each plate included eighteen exposures, for five of which standard spills were used. These were prepared of filter-paper without plant material, on to which standard solutions had been pipetted and allowed to dry. The volume delivered by the micropipette used was 0·0619 ml. A solution of metaphosphoric acid (0·0319M) was first added to each spill; after this first solution had dried, one of five other standard solutions was added. The most concentrated of these solutions contained:

0·208M calcium chloride,	0·00156M manganous chloride,
0·00743M ferric chloride,	0·328M potassium chloride,
0·133M magnesium chloride,	0·0905M sodium chloride,

and the others were 1/2, 1/4 and 1/10 dilutions of this, and distilled water. The five different standard spills were randomized among the thirteen spills containing plant material, and all were carbonized and burnt in succession. The spectra were recorded on Ilford Zenith plates, and development was under thermostatic control.

The lines used for photometric measurement were: calcium 422·6 mμ, iron 372·0 mμ, magnesium 285·2 mμ, manganese 403·1 mμ, potassium 404·4 mμ, sodium 330·3 mμ; the doublets for calcium and sodium and the triplet for manganese were not resolved. Before the commencement of measurements, the photocell was prefatigued for 15 min. and the photometer was then set to give a galvanometer deflexion of 50 cm. on a clear portion of the plate. Each spectrum being divided by the step-sector into four regions of different density, the portion of each line to be measured was selected, as far as possible, to give a galvanometer deflexion of

between 5 and 30 cm. at the point of maximum density. The lines on the standard spectra were, of course, measured at the same exposures as proved most appropriate for the spectra of plant material. In addition to the reading in the region of maximum density of each line, a reading for background was taken at 0·3 mm. to one side of this region (in the case of magnesium, the mean of readings on both sides of the line was used).

A separate working curve for each wave-length was prepared for the purpose of converting the galvanometer readings into estimated intensity values. In view of the difficulty in making known and gradual changes in the intensity of a light source, the working curves were prepared by finding the galvanometer readings for spectra obtained by exposure to a standard light source for increasing periods. In converting the galvanometer readings, the estimated intensity value for that in the centre of the line was first read from the working curve, then that for the background reading beside the line, and the difference gave the estimated intensity of the line proper (in arbitrary units). The estimated intensity values for the five standard spectra on the plate were then plotted against the amount of the element added, and a smoothed curve was drawn through the points. The amounts of the element in the samples of plant material burnt were then estimated from this curve by using the estimated intensity values obtained for them.

The accuracy of this method was assessed by preparing eight spectra of each of twelve samples of plant material, and comparing the results obtained with those from spectra prepared by means of the Lundegårdh apparatus. The means for all twelve samples, with the standard error of the difference between the mean values for the two methods, are shown in Table 1.

TABLE 1. *Mean composition of twelve samples of plant material as determined by two methods of flame spectrography*

	Mean Roach method	Mean Lundegårdh method	Standard error of difference between means
Calcium (%)	0·238	0·291	0·028
Iron (p.p.m.)	560	470	52
Magnesium (%)	0·132	0·156	0·013
Manganese (p.p.m.)	64·6	70·8	3·4
Potassium (%)	2·28	2·34	0·08
Sodium (%)	0·651	0·680	0·013

The arrangement of the spectra, together with repeated photometry, made it possible not only to estimate the error variance of a single observation, but also to find what part of this error variance was attributable to (*a*) the photometric process and subsequent operations, (*b*) differences arising within a single plate and (*c*) differences between plates, including of course those between their standard spectra. These results are shown in Table 2.

TABLE 2. *Error of spectrographic analysis by modified Roach method*

	Coefficient of variation of a single observation (%)	Percentage of error variance due to photometry, etc.	Differences within plates	Differences between plates
Calcium	31·6	2·7	70·7	26·6
Iron	19·7	20·7	74·5	4·8
Magnesium	24·1	4·9	63·4	31·7
Manganese	19·9	5·5	89·7	4·8
Potassium	16·0	8·3	83·1	8·6
Sodium	15·4	8·1	78·5	13·4

Analyses were performed at least in duplicate, the replicate spectra being recorded on separate plates.

RESULTS

The analytical data were expressed as percentages of the element in question in the dry matter. In view of the large range of variation in the data for most of the elements studied, it was necessary to convert all the percentages except those for magnesium into their logarithms before they could be analysed statistically. The means quoted for the other five elements are thus geometric means, and the differences whose significance is tested are proportionate differences. The differences among the five types of sample from each plot were treated as 'subplots' in the analysis, and the unconfounded interactions between fertilizer treatments were used as the error estimates. Both plot and subplot error estimates were examined by Bartlett's (1937) μ-test, and found to be uniform as between the four sites with the exception of the subplot error for iron and magnesium, and the plot error for calcium and iron. The significance of comparisons between sites was tested by the methods described by Yates & Cochran (1938).

Calcium. The mean content of calcium in each organ at each site is shown in Table 3. The differences between means for sites and for organs are significant, and so is their interaction ($F = 2{\cdot}12$, $n_1 = 12$, $n_2 = 315$). This significant interaction derives from the much greater proportional differences in calcium content in the lower leaf blades than in the other organs.

TABLE 3. *Mean calcium content (percentage calcium in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	0·291	0·184	0·187	0·096	0·095	0·156
Kimpton	0·582	0·184	0·219	0·136	0·103	0·201
Leckford	0·373	0·220	0·215	0·120	0·088	0·179
Snoddington	0·594	0·233	0·224	0·136	0·094	0·209
All	0·440	0·204	0·211	0·121	0·095	0·185

The only treatment effect reaching significance in the calcium data is the interaction of ammonium sulphate application with organ differences at Chilbolton

($F=2{\cdot}21$, $n_1=8$, $n_2=80$). The relevant means are presented in Table 4. It should, however, be pointed out that this interaction is only barely significant, and may perhaps be regarded as fortuitous.

TABLE 4. *Effect of ammonium sulphate on calcium content of organs of barley plants at Chilbolton*

Sulphate of ammonia (lb./acre)	Calcium content (% Ca in dry matter) of				
	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears
0	0·217	0·186	0·147	0·093	0·091
84	0·349	0·115	0·231	0·105	0·107
168	0·326	0·290	0·194	0·092	0·088

Iron. The mean content of iron in each organ at each site is shown in Table 5. The differences among organs, and between Kimpton on the one hand and Leckford and Chilbolton on the other, are significant. The plot error at Snoddington was exceptionally large, so the differences between the mean iron content at this site and the values for the other three are not significant. The interaction between organs and sites (weighting values from different sites inversely as the error variance estimates) was also highly significant, this being due to the anomalous results at Snoddington, where the iron content of the sheaths was unexpectedly high, that of the young leaves and ears unexpectedly low.

TABLE 5. *Mean iron content (p.p.m. iron in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	189	100	136	83	126	122
Kimpton	261	157	194	101	179	171
Leckford	204	92	138	82	113	119
Snoddington	269	178	129	95	122	148
All	228	127	147	90	133	138

The only significant effects of the fertilizer treatments on iron content were at Chilbolton, where muriate of potash had depressed the mean iron content from 140 to 121 p.p.m. with the single application and 108 p.p.m. with the double application, and at Kimpton, where the interaction of potassic fertilizer with the organ differences was significant ($F=2{\cdot}96$, $n_1=8$, $n_2=77$); the treated plots here had a higher iron content in young leaves and stems, but lower values in sheaths and ears, than the untreated plots (Table 6). It is worth noting that at Leckford and Chilbolton the unusual result was observed that the subplot error variance was greater than that for the plots ($F=2{\cdot}03$ and $2{\cdot}55$ respectively, $n_1=80$, $n_2=18$).

Magnesium. In Table 7 are given the magnesium content values for the different types of sample at each site. Organ differences, site differences, and their interaction

TABLE 6. *Effect of potassic fertilizer on iron content of barley plants at Kimpton*

Muriate of potash (lb./acre)	Iron content (p.p.m. Fe in dry matter) of Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears
0	249	205	177	87	208
56	277	156	199	88	163
112	258	121	208	133	170

TABLE 7. *Mean magnesium content (percentage magnesium in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	0·215	0·123	0·180	0·126	0·160	0·161
Kimpton	0·258	0·139	0·182	0·134	0·174	0·178
Leckford	0·216	0·150	0·163	0·152	0·152	0·167
Snoddington	0·211	0·120	0·135	0·120	0·172	0·152
All	0·225	0·133	0·165	0·133	0·165	0·164

are all significant. The significance of the interaction appears to depend upon the fact that, whereas the figures from Kimpton are highest for most organs, Leckford usurps the first place for the two organs—sheaths and stems—containing a high proportion of conducting and mechanical tissue. The implications of this are obscure.

The only treatment effects to reach significance are the mean effects of the muriate of potash application, no interaction with the organ differences being found. The mean effects at each site are shown in Table 8. The difference between the means for the plots receiving the double application and the control plots is highly significant ($F=32{\cdot}41$, $n_1=1$, $n_2=3$); the value for the plots receiving a single application does not differ significantly from the mean of the remainder, and the interaction of treatment with location is non-significant.

TABLE 8. *Effect of potassic fertilizer on magnesium content (percentage magnesium in dry matter) of barley plants*

Muriate of potash (lb./acre)	Chilbolton	Kimpton	Leckford	Snoddington	All locations
0	0·180	0·184	0·178	0·164	0·177
56	0·163	0·190	0·170	0·143	0·166
112	0·140	0·159	0·153	0·147	0·150

Manganese. The mean values for manganese content of the plants at the four sites are shown in Table 9. The organ means differ significantly, and the mean value for Kimpton differs significantly from those at the other sites. There are also significant interactions, both between Kimpton and the other sites ($F=3{\cdot}93$, $n_1=4$, $n_2=315$) and among the other sites ($F=3{\cdot}99$, $n_1=8$, $n_2=315$). The significance of the interaction between Kimpton and the other sites depends upon the greater

proportionate difference in the manganese content of the young leaves than in that of the other organs tested ($F = 13{\cdot}45$, $n_1 = 1$, $n_2 = 315$). The significance of the interaction among the sites other than Kimpton appears to be due to the anomalously low manganese content of the stems at Leckford ($F = 18{\cdot}54$, $n_1 = 1$, $n_2 = 315$).

TABLE 9. *Mean manganese content (p.p.m. manganese in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	125	90	61	58	31	66
Kimpton	171	124	91	61	38	85
Leckford	133	91	61	43	30	62
Snoddington	137	96	52	53	29	64
All	140	100	65	53	32	69

Among treatment effects, the mean effect at all sites of the muriate of potash applications in reducing the manganese content of the plants (Table 10) is significant ($F = 10{\cdot}56$, $n_1 = 2$, $n_2 = 6$). The differences among the sites in this respect do not quite reach significance ($F = 2{\cdot}10$, $n_1 = 6$, $n_2 = 72$), but the mean interaction with organ differences over all four sites is significant ($F = 3{\cdot}16$, $n_1 = 8$, $n_2 = 24$). The details are shown in Table 10. This significant interaction arises from the greater proportionate effect of the fertilizer on the manganese content of the upper leaves than on that of any of the other organs (comparing the upper leaves with the lower leaf blades, which among the other organs, show the greatest differences, $F = 6{\cdot}33$, $n_1 = 2$, $n_2 = 6$).

TABLE 10. *Mean effect of potassic fertilizer on manganese content (p.p.m. manganese in dry matter) of different parts of barley plants, at all sites*

Muriate of potash (lb./acre)	Manganese content of: Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All parts
0	155	109	79	55	34	76
56	137	93	61	55	31	67
112	130	97	57	51	30	64

Potassium. Since the sites for these experiments were selected on grounds of suspected potassium deficiency, the greatest interest attached to the analyses for this element, and more marked differences in its concentration in the plants were to be expected than for the other elements. The mean values for the four sites are given in Table 11. Site and organ differences and their interaction are all significant. The major part of the significant interaction arises from the fact that the differences in the ears are in the inverse direction from those in all other organs—Leckford having the lowest potassium content in the ears and the highest in the other organs, whereas Kimpton has the lowest values for most organs but the highest for ears.

Among the vegetative organs, the interaction of the difference between lower leaf blades and sheaths with site differences is not significant, but the proportionate differences between sites in these two organs are significantly greater than those in stems ($F=4{\cdot}92$, $n_1=3$, $n_2=315$) or young leaves.

TABLE 11. *Mean potassium content (percentage potassium in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	0·64	0·58	1·48	0·88	2·23	1·02
Kimpton	0·55	0·58	1·34	0·74	2·51	0·95
Leckford	1·04	1·00	1·72	1·09	1·86	1·30
Snoddington	0·90	0·79	1·81	1·04	2·36	1·26
All	0·76	0·72	1·58	0·93	2·23	1·12

The mean effects of muriate of potash on the potassium content of the plants from the different sites are shown in Table 12. The mean effect of the treatment is highly significant ($F=35{\cdot}15$, $n_1=2$, $n_2=6$), and the proportionate effects of the successive additions of 56 lb. do not differ significantly from one another, or between the different sites, except at Leckford, where the effect of the second 56 lb. is negligible.

TABLE 12. *Effect of potassic fertilizer on mean potassium content of samples from different locations*

Muriate of potash (lb./acre)	Mean potassium content (% K in dry matter) at				
	Chilbolton	Kimpton	Leckford	Snoddington	All locations
0	0·82	0·66	0·9	0·96	0·83
56	0·99	0·95	1·54	1·29	1·17
112	1·30	1·37	1·57	1·58	1·45

TABLE 13. *Effect of potassic fertilizer on mean potassium content at all sites of different organs*

Sulphate of potash (lb./acre)	Mean potassium content (% K in dry matter) of				
	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears
0	0·51	0·46	1·09	0·68	2·23
56	0·80	0·73	1·82	0·96	2·20
112	1·07	1·09	1·99	1·22	2·26

The fertilizer has not affected the potassium content of the different organs uniformly (see Table 13). The significant interaction here arises entirely from the constancy of the potassium content of the ears, in contrast with the very considerable increases observed for the other organs ($F=30{\cdot}33$, $n_1=2$, $n_2=6$). The only other

main treatment effect to reach significance is that of superphosphate at Kimpton ($F=4{\cdot}83$, $n_1=2$, $n_2=18$):

Superphosphate (lb./acre)	Mean potassium content of all organs (% K in dry matter)
0	1·10
168	0·91
336	0·86

In general the significance of interactions among the fertilizer treatments was not tested, and any such possible effects were included in the error estimate. In the case of the data for potassium content, however, since the effects of the application of sulphate of potash were so large and the main effect of superphosphate was significant at one site, it was considered desirable to test the significance of the first-order interactions. It was found that the mean interaction between sulphate of ammonia and superphosphate at all sites was in fact significant ($F=5{\cdot}53$, $n_1=4$, $n_2=12$). As shown in Table 14, this depended upon the fact that, whereas in combination with sulphate of ammonia superphosphate had the effect of diminishing the potassium content of the plants, where no sulphate of ammonia was applied the tendency was in the opposite direction.

TABLE 14. *Mean effects of sulphate of ammonia and superphosphate treatments on potassium content (percentage potassium in dry matter) of all organs at all locations*

Superphosphate (lb./acre)	Sulphate of ammonia (lb./acre)		
	None	84	168
0	1·04	1·23	1·17
168	1·11	1·10	1·20
336	1·15	1·07	1·03

Sodium. In Table 15 are given the data for mean sodium content of the different organs at each location. Differences between sites and organs, and their interaction, are significant. The significant interaction ($F=10{\cdot}89$, $n_1=12$, $n_2=315$) derives principally from the greater proportionate differences in the upper leaves than in the other organs—the differences are significantly greater even than those in the ears, otherwise the most variable part ($F=4{\cdot}02$, $n_1=3$, $n_2=315$).

TABLE 15. *Mean sodium content (percentage sodium in dry matter) of each organ at each location*

Location	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
Chilbolton	1·175	1·443	0·581	1·153	0·159	0·710
Kimpton	0·838	1·144	0·338	0·717	0·120	0·489
Leckford	0·459	0·671	0·114	0·547	0·047	0·246
Snoddington	0·761	0·825	0·187	0·726	0·063	0·351
All sites	0·766	0·978	0·255	0·757	0·086	0·407

The effect of muriate of potash on the sodium content of the plants, and its interaction with organ differences, prove to be significant at every site, and at all sites considered together (see Table 16). Though the sodium content falls with increasing potassium supply, the proportionate decrease differs from organ to organ ($F = 4{\cdot}12$, $n_1 = 8$, $n_2 = 24$), being greatest in the young organs and least in the stems. The differences between the effects of the two successive additions of fertilizer are not significant.

TABLE 16. *Mean effects of potassic fertilizer on the sodium content (percentage sodium in dry matter) of different organs*

Muriate of potash (lb./acre)	Sodium content (% Na in dry matter) in					
	Lower leaf blades	Lower leaf sheaths	Upper leaves	Stems	Ears	All
0	0·908	1·294	0·405	0·863	0·118	0·544
56	0·755	0·917	0·228	0·694	0·088	0·395
112	0·655	0·787	0·179	0·725	0·062	0·334

Relationship between potassium content and response to potassic fertilizer

As has been indicated, the main purpose of this investigation was to ascertain whether analysis of barley plants for potassium was likely to give information as to the adequacy of the potassium supplies at their disposal, and, if so, of what part of the plant was analysis likely to be most informative. The most practical criterion as to whether supplies of a nutrient to a plant are adequate or not is whether it responds to additional supplies of it by an increased yield. For eighteen of the twenty-seven plots at each of the four sites studied, an estimate was available of the yield increase which would have resulted from an application of 56 lb./acre of muriate of potash, in the differences in yield of grain between the plots receiving 56 or 112 lb./acre on the one hand, and 0 or 56 lb./acre on the other, but the same amounts of superphosphate and sulphate of ammonia. Since, however, plots whose treatment differed only in respect of potassium fell into different blocks, as a result of the confounded design, and the block means differed significantly, it was necessary to correct the difference in yield between the pair of plots for the block difference. Thus there was available a series of seventy-two estimates of the increase in grain yield as a result of the application of 56 lb./acre of sulphate of potash, which could then be compared with the potassium content of the material from the plot with the lower level of treatment—the analytical data being corrected for block differences in the same way as the yield data.

At Leckford and Snoddington, none of the correlations, between response on the one hand and potassium content of the various organs on the other, was significant. At the other two sites, however, several of the correlations were significant, and these, together with those resulting from combining the values within all four sites, are indicated in Table 17. Apart from the fact that the ears do not appear in this

table, and hence are presumably to be rejected for diagnostic purposes, these coefficients do not give a clear picture enabling a preference to be expressed between one organ and another. It must be noted that in most cases the differences among the correlation coefficients shown are quite non-significant, and indeed that most of them do not differ significantly from many of the other non-significant coefficients not shown in the table.

TABLE 17. *Regression of yield response (cwt. grain per acre) to single application of potassic fertilizer on* $\log_{10}$ *(percentage potassium in dry matter) on individual plots within sites*

Location	Organ	r	b	P
Chilbolton	Lower leaf blades	−0·475	−17·05	0·02–0·05
	Upper leaves	−0·547	− 8·21	0·01–0·02
	Stems	−0·536	−16·10	0·02–0·05
Kimpton	Lower leaf sheaths	−0·685	−15·86	0·001–0·01
	Upper leaves	−0·514	− 8·04	0·02–0·05
All locations	Lower leaf blades	−0·271	− 4·34	0·02–0·05
	Lower leaf sheaths	−0·248	− 3·62	0·02–0·05
	Upper leaves	−0·406	− 6·10	<0·001

It might well be argued that, for practical diagnostic purposes, any correlations between these variates which may appear in the means for the four sites would be of greater importance than the correlations within sites given in Table 17. As will be seen from Table 18 the correlations of yield response at different locations with the potassium content of the lower leaves and stems are in fact significant, and those with the potassium content of the lower leaf blades and of the stems are significantly greater than those for any of the other organs, though they do not differ significantly from one another.

TABLE 18. *Regression of mean yield responses (cwt. grain per acre) to single application of potassic fertilizer on mean* $\log_{10}$ *(percentage potassium in dry matter) at different sites*

Organ	r	b	P
Lower leaf blades	−0·999955	−12·37	<0·001
Lower leaf sheaths	−0·9578	−13·78	0·02–0·05
Upper leaves	−0·9320	−20·30	0·05–0·10
Stems	−0·999793	−18·89	<0·001
Ears	+0·7358	+26·98	>0·10

These relationships between potassium content and response to potassic fertilizer are presented graphically Figs. 1–4. The mean values for each site are shown, but not those for the individual plots. The lines represent such of the regressions as are significant—the broken lines those between site means, the full lines those between plots at individual sites. The potassium content of the two organs for which the correlation is greatest, corresponding on the basis of the regression between sites to zero response is: lower leaf blades 0·924%, stems 1·014%.

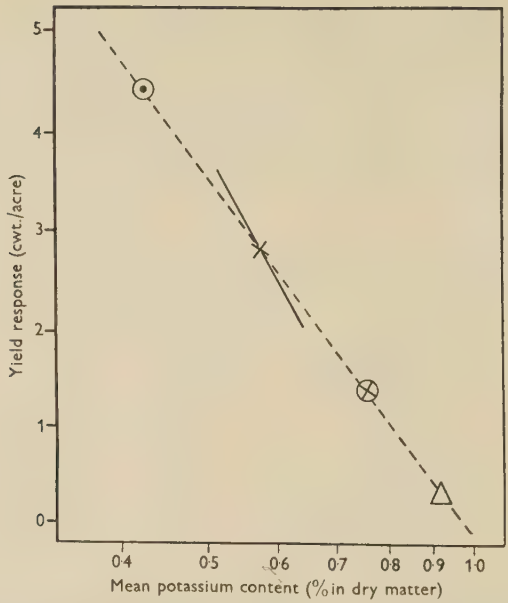

Fig. 1. Regression of response (increase in grain weight) to single dressing of muriate of potash on potassium content of lower leaf blades.

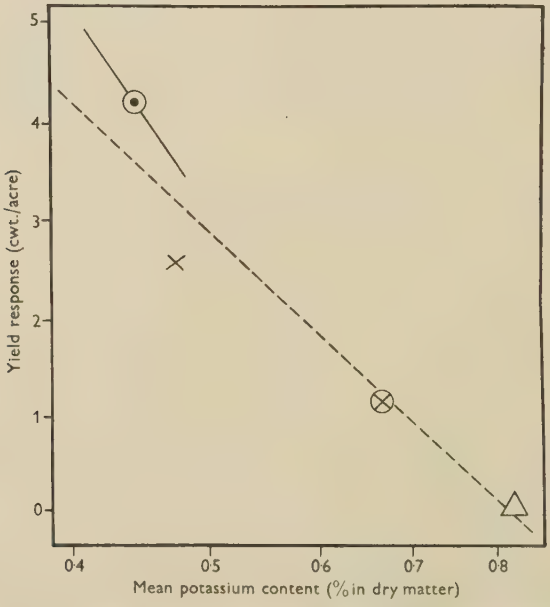

Fig. 2. Regression of response (increase in grain weight) to single dressing of muriate of potash on potassium content of lower leaf sheaths.

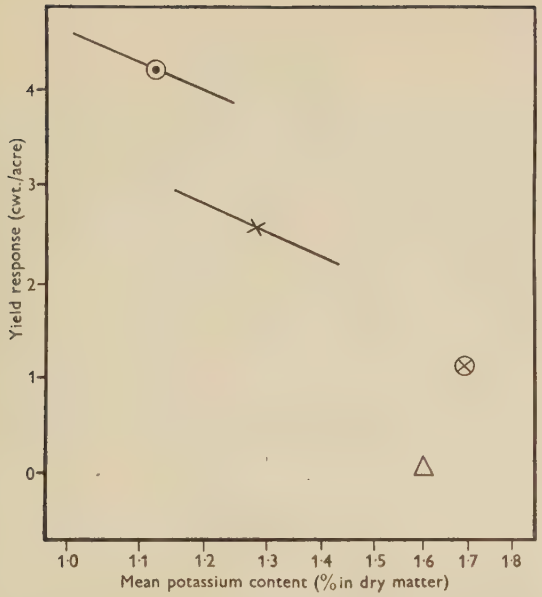

Fig. 3. Regression of response (increase in grain weight) to single dressing of muriate of potash on potassium content of young leaves.

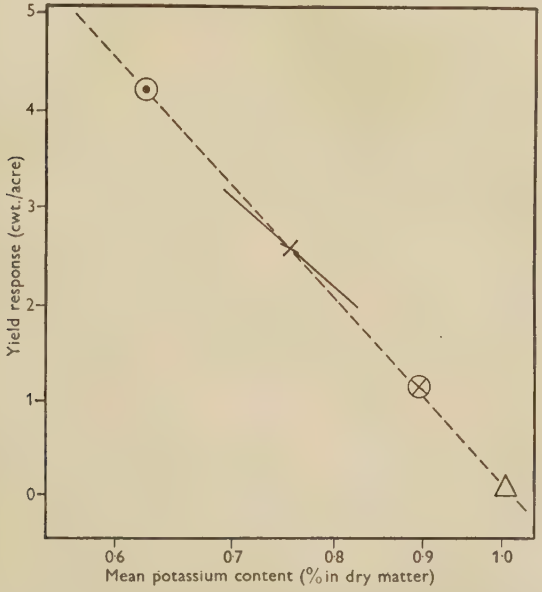

Fig. 4. Regression of response (increase in grain weight) to single dressing of muriate of potash on potassium content of stems.

Discussion

As was to be expected, the main treatment effects observed in the investigations were those resulting from the application of muriate of potash. This treatment increased the mean content of potassium in the plant, but decreased that of magnesium, manganese and sodium. It is, of course, very general for an increase in the supply of one cation to result in an increase of its concentration within the plant, and a decrease in that of other cations. As examples of such an effect of potassic fertilizers on the magnesium content of cereals may be cited the observations of Godlewski (1901), Münter (1919), Loehwing (1928), Thomas and Mack (1939) and Stanford *et al.* (1942). A decrease in manganese content of sugar beet as a result of application of potash fertilizers was reported by Hale *et al.* (1946). In the present data, the sodium content of the plants seems to show an inverse relationship with the potassium content; this is evident not only in the effects of the potassic fertilizer on their composition, but also in the differences in composition between different organs and sites (with the exception of the comparison between Chilbolton and Kimpton; in this connexion, it may be noted that the calcium and magnesium contents of the samples from Kimpton were particularly high).

The nitrogen and phosphorus fertilizer treatments have had rather little effect on the internal concentration of the elements studied. The absence of an increase in manganese content as a result of ammonium sulphate application is particularly noteworthy in view of the frequency with which such results have been reported (Goodall, 1943).

For every element, the internal concentration differs markedly from organ to organ. It may be noted that the young ears have a very high potassium content, which remains unaffected by differences in potassium nutrition. It varies somewhat from site to site, in the opposite direction to that of the vegetative organs harvested; possibly these differences in potassium content of the ears may be due to slight differences in the stage of development at the time of harvest, or to different proportions between the parts of the ears at the different sites. The calcium, manganese and sodium concentration in the ears, on the other hand, are all notably low. In the stems, the content of every element but sodium is low, and the same is true of the sheaths with the further exception of manganese; these results are doubtless associated with the high proportion of conducting and mechanical tissue in these organs.

If the upper and lower leaves are compared, it will be seen that the former have a higher concentration of potassium, the latter of all other elements. This difference can doubtless be ascribed in part to the great mobility of potassium within the plant, for it can readily be translocated from an older organ to meet the needs of growing tissues. In connexion with this question of the mobility of elements within the plant, it is worth drawing attention to the observation that at two sites the subplot error for iron was greater than the plot error. This result is fully parallel with one

reported earlier for apple leaf blades and petioles (Goodall, 1945); the most probable explanation appears to be that there is within the subplot error a negative correlation between the iron concentration in different organs of the same plant; in other words, some factors other than those under experimental control have in certain plants caused iron to be retained in a non-mobile form in the organs with abundant conducting tissue, so that they have an abnormally high, and the distal organs an abnormally low, iron content.

The principal purpose of the present investigation was to aid in the selection of a type of sample for diagnostic analysis. For potassium, data on the response to potassic fertilizers being available, the accuracy with which this response could be forecast from the analytical data provides the best criterion for the selection of a sample type. Comparisons between plots within sites enable one to reject the ears for this purpose, but do not give very satisfactory grounds for choosing between the vegetative organs harvested. Comparisons between site means, however, indicate that the lower leaf blades or the stems are likely to enable a more reliable forecast to be made than the sheaths or upper leaves. The potassium content of the older leaf blades is, moreover, rather more sensitive to differences in potassium status in or between sites than that of the stems (see Tables 11 and 13), so the lower leaf blades must be indicated as the most promising type of sample for the diagnosis of potassium deficiency at the stage of ear emergence.

In respect of calcium, manganese and sodium content, too, there are clear differences between the organs in sensitivity to site differences. For calcium, the site differences are most marked in the lower leaf blades, for manganese and sodium in the upper leaves. It would, however, be somewhat dangerous to draw from these facts conclusions as to the diagnostic value of the different types of sample for deficiencies of these elements. Whether the differences in composition reflect differences in the availability at the various sites of the element in question is open to doubt—in the case of manganese and sodium, the differences appear to be associated inversely with potassium status. In wheat, the author has found (Goodall, 1949) that the manganese content of the lower leaves is a more sensitive index of manganese deficiency than that of the upper leaves; from the present results, it would seem quite possible that differences in manganese content induced by differences in potassium supply may, on the other hand, be more marked in the upper leaves.

It is clear that the work reported in this paper is no more than preliminary and tentative. In the first place, samples were taken at one stage of development only, and that too late for the remedy during the current season of any deficiencies discovered. In this connexion, it should be mentioned that Maume & Dulac (1934, 1934*a*, 1935, 1935*a*, 1936, 1936*a*, 1937, 1938, 1938*a*, 1942) gathered plants at *épiaison* for diagnostic purposes, but considered it very important to harvest at precisely the same stage of development, since the composition of the plants was changing rapidly; on the other hand, Burström (1937) selected this stage because, according to his data, the rate of change in mineral content was *low* at the

time. The rapid changes found by Maume & Dulac may well have been due to the fact that they harvested and analysed the whole aerial part of the plant, in which of course the proportions of the different organs were changing rapidly, whereas Burström was concerned only with the leaves; Gregory & Richards (1929) found relatively small changes in successive analyses of the same leaf of barley during this period of development, though Richards (1932) subsequently found greater changes in those of his treatments which were severely deficient in potassium. It is clear that for the present investigation, had it been possible, it would have been preferable to carry out a series of successive harvests from an early stage of development. A second respect in which this work is in need of extension is that it was confined to a particular group of soils, and to a single year. It is evident that only with considerable reserve could the results be extended to other soil types and to the meteorological conditions of other seasons. Variety too may play some part in the relationships observed.

With the restrictions embodied in the above comments in mind, it may be of interest to compare the limiting values for composition of leaves and stems cited here with other similar values reported in the literature. Of those quoted in the introduction, only those of Atterberg (1888) and Lundegårdh (1938, 1939, 1941, 1943), both with oats, appear to be comparable with the present data, for most other investigations involved analysis of samples taken at maturity. Atterberg found that, as potassium deficiency became more severe, the potassium content of the entire aerial parts harvested at flowering time approached 0·31 % K_2O, while Lundegårdh concluded that in samples of the leaves taken between panicle emergence and flowering a good response to potassic fertilizer was to be expected when the potassium content was not more than 30 m.equiv. % (1·17 % K), the phosphorus status being average. This figure of Lundegårdh is substantially greater than that obtained for the older leaf blades of barley at ear emergence in the present work. But Lundegårdh's samples included leaves of all ages, and since the potassium content of the younger leaves is greater than that of the older ones the two values may be taken as reasonably consistent. The responses to superphosphate were significant only at Chilbolton and Snoddington, amounting on an average to about 2 cwt. of grain per acre for the double application, so these sites would probably fall into Lundegårdh's average phosphate category. The potassium content of the barley samples was in all cases well above Atterberg's minimum level for oats at flowering time.

The author wishes to acknowledge his appreciation of the interest shown in this work by Professor F. G. Gregory, Dr R. G. Hatton, and Dr W. A. Roach. His thanks are also due to Mr M. N. Nicholson, the Advisory Chemist for the Southern Province, and to the Hampshire War Agricultural Executive Committee and its officers, for the opportunity to gather samples from their fertilizer trials, and for the provision of the yield data used. Miss E. M. Ford, Miss B. Kidwell, Mr C. King, Mrs J. King, Mr J. H. Lavender and Miss D. Martin assisted in the preparation of

the samples and in their subsequent analysis. A large part of the spectrographic apparatus used was purchased with the aid of funds made available to Dr W. A. Roach by the Government Grants Committee of the Royal Society.

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GROWTH CYCLE METABOLISM OF PLANTS IN RELATION TO FLOWERING

By JOHN GRAINGER
Department of Plant Pathology, West of Scotland Agricultural College, Auchincruive, Ayr

(With 7 Text-figures)

An examination of the percentages of total carbohydrate, nitrogen (Kjeldahl) and ash has been made at various times in the growth cycle in six plants of varying growth habit. The time of formation of the flower initial in four species (fumitory, leek, radish and yellow water-lily) corresponds to a maximum in the curve showing percentage of total carbohydrate plus percentage of ash in the whole plant. The other two species (pea and bean) make their flower initials very early in the growth cycle, at a time when the amounts of carbohydrate plus ash are high because of the attachment of the plant to the large seed. These two species therefore also make their flower initials at a virtual maximum of carbohydrate plus ash. All six species of plants studied have mobile carbohydrate. The distribution of nutrients after flowering is discussed.

Many studies have been made into the various factors which affect flowering. Some exert a minor effect, in hastening or retarding the appearance of bloom. Others affect floral organization in a major, determinative way; they have an influence upon the actual change from the vegetative to the flowering state. Studies upon photoperiod and phasic development have shown the magnitude of certain factors of environment acting at particular times in the growth cycle. There is as yet, however, no clear picture of the mode of action within the plant of these specific effects of environment. It seems reasonable to suppose that the actual transfer from vegetative growing point to flower initial would coincide with, or follow, a change in physiology or nutrition of the plant. Can any changes in the major nutrients or the hormonal system be correlated with flower initial production? The present investigation attempts to answer the nutritional part of this question.

Previous work

The classical attempt to link nutrition and flowering is that of Kraus & Kraybill (1918) following earlier indications by Klebs. It was suggested that the tomato plant flowered when the ratio between carbohydrate and nitrogen attained a certain value. This postulate seemed to explain various horticultural practices, but it does not always apply. Sheard (1940), for example, found that flower initial formation in chrysanthemum and other plants did not invariably coincide with a high C/N ratio.

Garner & Allard (1920) demonstrated the major effect of the daily duration of light upon flowering. Certain plants flowered only during the short days of autumn, whilst others bloomed during the long days of summer. The 'short-day' plants

could be made to flower in summer if the daily period of light were artificially curtailed. Work by the writer (Grainger, 1938) with late-flowering chrysanthemum, suggested that this short-day plant can only flower when nights are long, because of a delay in nocturnal translocation of carbohydrate. The short nights of summer only allow very limited translocation, but if they are lengthened artificially, more carbohydrate is translocated in the longer period of darkness, and the plant flowers. Water plants flower in Britain during the longest days of summer. It has been shown (Grainger, 1947) that submerged foliage suffers a nocturnal loss of carbohydrate (probably into the surrounding water), which is not sustained if the leaves are exposed to the air. The shorter the night, the smaller the loss from under-water leaves, and the higher the carbohydrate level. Flowering round midsummer therefore coincides with the highest amounts of carbohydrate of any period of the year. Submerged plants of *Littorella* and *Callitriche*, moreover, remain vegetative with a low carbohydrate content, but flower quickly when exposed to air, when the carbohydrate increases markedly. This all seems to indicate the amount of carbohydrate available to the growing point as a dominant factor in flower initiation. It cannot be the sole factor, however, for mineral nutrients can affect flowering (e.g. Loehwing, 1940), and nitrogen has a particular effect. Čaĭlachjan (1945) has, in fact, classified plants as 'nitropositive', which flower earlier with normal or increased nitrogen, 'nitronegative', flowering more quickly with reduced nitrogen or starved of it, and 'nitroneutral', which flower at times not affected by the nitrogen supply. The amount of nitrogen, therefore, cannot invariably be a major factor in flowering. Micro-nutrients can also affect the time of flowering; boron, for instance, caused earlier flowering (curd) of cauliflowers, when compared with growth made with insufficient supplies of this element (Dennis & O'Brien, 1937). It would seem, however, that inorganic nutrients do not affect floral initiation as potently as does the supply of carbohydrate.

It seems possible that the effects of photoperiod upon plants might also be explained on a 'floral stimulus' or hormonal basis (e.g. Čaĭlachjan, 1937; Hamner & Bonner, 1938; Cholodny, 1939; Moshkov, 1937; Withrow & Withrow, 1943). This does not seem to provide a valid reason for not investigating the direct nutritive aspects of flower initiation. Even if flowering is controlled by hormones, there is no reason why control should not be exerted through, or reflect, an effect upon balance or amount of nutrients.

Lysenko and other Russian workers, following earlier investigations by Klebs and Gassner, formulated the idea of vernalization. This was later crystallized into a more specific concept of phasic development: a plant, in its early stages, is subject to the inductive effects of temperature and length of day, so far as its ultimate flowering reactions are concerned. An adequate review of modern developments in this field, of the work in this country by Gregory and Purvis, and of numerous American investigations, is given by Whyte (1946). Manipulation of the appropriate factor of environment in each phase affects the rate of development or the

time of flowering. It is not clear, as yet, whether such manipulations have any direct determinative effect upon the transition from vegetative to floral organization of the growing point. They appear to dictate the length of time spent in any particular phase of development, and so may have an indirect effect. The present investigation is concerned with internal conditions associated with the change from vegetative to floral symmetry of the growing point of normal, non-vernalized plants. It would seem to have a logical sequel in a comparison of growth cycle metabolism of vernalized and unvernalized plants.

METHODS

Studies were made upon six plants of widely differing type. Large beds of each species were established, so that whole plants could be removed successively at different stages in their cycle of growth. They were subdivided into root, shoot, flower, fruit, etc., and were preserved for analysis by heating to 98° C. for half an hour, followed by drying to constant weight at 65° C. Each sample was powdered by grinding in a mortar, or by passage through a small hammer mill. Total carbohydrate was estimated by the method of Willaman & Davidson (1924); total organic nitrogen was measured by means of a semi-micro Kjeldahl method adapted from Pregl (1930), ash was determined by heating in a muffle furnace, and fat, where necessary, by a small-scale Soxhlet determination. Fumitory, pea, bean and leek were grown at or near the Tolson Memorial Museum, Huddersfield, in 1943. Yellow water-lily was obtained from Canon Hall Park, Cawthorne, near Huddersfield, in 1943. Radish was grown at Auchincruive in 1944. Each sample of plants contained a varying number, larger in the early stages of growth, and smaller when mature. Figures for dry weight of various plant parts are averages from these numbers, and in general, not less than six plants were taken, even when mature. Analyses were made on bulked samples, i.e. from the appropriate parts of all plants in the sample, ground together. Difficulties of sampling and separation were greater for yellow water-lily than for the other plants, and the samples in this case did not usually consist of more than two plants.

Samples were also taken from leaves of each plant at intervals through a major part of a 24 hr. period. These were examined for the amount of total carbohydrate, in order to estimate its mobility.

Examination for flower initials was by direct dissection under a × 10 lens, or low-power binocular microscope, whichever was necessary. Plants were examined for the presence of flower initials more frequently than they were sampled for analysis, so the times given for floral initiation may be taken as those when flower primordia were first recognizable under a low-power microscope.

Figures for the percentage and amounts of dry matter, total carbohydrate, ash and nitrogen in the whole plant have been calculated by adding the amounts of each fraction in all parts, and determining percentages from these sums. This avoids the introduction of weighted means.

Mobility of the carbohydrate

Fig. 1 shows that there is, during the night, a fall of total carbohydrate in leaves of pea (A), leek (B), fumitory (C), bean and radish (D). This has been taken as an indication of mobility of the carbohydrate. Floating leaves of the yellow water-lily have similar nocturnal loss (Grainger, 1947). This survey shows that the plants here studied are all of one type, having ready translocation of carbohydrate, without any disabilities such as those mentioned above for short-day plants and aquatic vegetation.

Results of the analyses

Tables 1–4 and Figs. 2 and 3 portray the salient features of the results of the analyses and also indicate the times of formation of flower initials. More results have been obtained than can be included in this paper. It does not seem necessary to give these *in extenso*; there is an essential similarity about them, and only such features, mostly relating to the whole plant, as have bearing on the subject of this paper have been included. A copy of the full results is held by the author, and another is available in the General Library of the British Museum (Natural History), Cromwell Road, London, S.W. 7. These can be consulted if desired.

It is necessary to distinguish between plants with large seeds (pea and bean) and those with small seeds (fumitory, radish, leek). The bean makes its flower initials before the young shoot emerges from the soil, and the pea shoot is florally determined very soon after the shoot reaches the air. Smaller-seeded plants have a period of purely vegetative growth before flower formation commences. This difference doubtless reflects the greater amounts of food available in the larger seeds.

Details of the accumulation of total carbohydrate, ash and nitrogen in these two types are shown by Figs. 2 (bean) and 3 (leek) which also portray the increase in dry weight. These two diagrams are generally typical of the other plants here examined, except water-lily, which represents a special case as an aquatic perennial. Increase in dry weight of the bean is plotted logarithmically, while that of the leek is plotted arithmetically. One noticeable feature of the leek is the greater amounts of dry weight, total carbohydrate, ash and nitrogen in the flowering plant, as compared with the vegetative individual. The superiority in dry weight is also apparent in the later stages of growth, as seen from Table 6, which refers to second-year plants sown in the spring of 1942.

Discussion

Possible relation between nutrition and flower initiation

In an attempt to trace any effect which the major food substances of the plant might have on the change from vegetative to floral organization, many correlations were tried. It seems reasonable to examine such changes in annual or biennial plants only up to the emergence of the flower, since a plant forming fruits achieves a concentration of food substances in them quite different from earlier distributions (see below and Fig. 6). The yellow water-lily provides a special case; it is a perennial

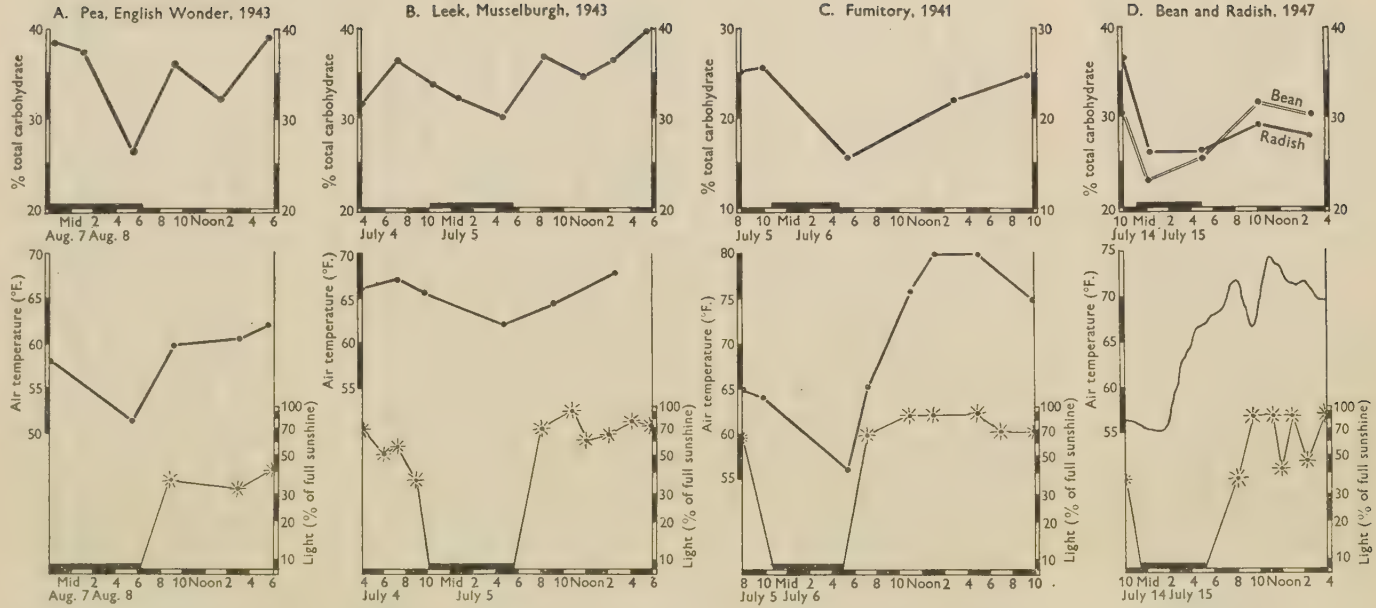

Fig. 1. Diurnal carbohydrate metabolism in five plants, with air temperatures and light readings. The upper graph for each plant shows the percentage of total carbohydrate, on dry weight basis, in leaves of that plant. A substantial fall in carbohydrate during the night is shown by all species, and this is taken as evidence of carbohydrate mobility. Air temperatures in A, B and C are from mercury thermometer readings; those in D are transcribed from a bimetallic recording thermograph. Light readings are from a Rex photoelectric exposure meter expressed on a logarithmic scale as an approximate percentage of full sunshine.

TABLE 1. *Fumitory*; *seed sown* 1 *December* 1942. *Data for the whole plant*

Date	Av. dry weight (g.)	% solids*	% total carbohydrate†	% fat†	% carbohydrate equiv.‡	% ash†	% N†
1 Dec.	0·004	—	16·66	18·33	62·49	4·52	2·40
4 Apr.	0·0077	15·27	24·42	—	—	12·24	4·00
11 Apr.	0·014	14·96	25·91	—	—	13·78	4·58
18 Apr.	0·033	13·58	23·18	—	—	16·84	5·24
2 May	0·093	13·46	25·10	—	—	16·98	5·22
9 May	0·145	11·68	22·57	—	—	18·10	5·68
23 May	0·831	12·18	27·24	—	—	15·17	4·75
20 June	6·70	12·34	32·04	0·46	33·20	11·56	3·55
4 July	7·91	19·22	30·30	7·36	48·70	10·91	3·13
18 July	8·36	22·77	31·35	4·14	41·70	12·58	2·11

* As percentage of fresh weight.
† As percentage of dry weight.
‡ Calculated as the percentage of carbohydrate plus 2½ times the percentage of fat.
Data giving times of production and numbers of flower initials are given in Figs. 4 and 7.

TABLE 2. *Pea, English Wonder*; *seed sown* 25 *April* 1943. *Data for the whole plant*

Date	Av. dry weight (g.)	% solids*	% total carbohydrate†	% ash†	% N†	Av. no. flower initials	Av. no. expanded flowers	Av. no. fruits
25 Apr.	0·183	90·00	57·21	3·64	2·61	—	—	—
16 May	0·178	7·38	30·16	8·42	5·61	—	—	—
23 May	0·20	9·24	28·57	11·36	5·09	—	—	—
30 May	0·28	—	21·73	16·80	4·38	1	—	—
6 June	0·475	11·11	20·00	16·68	4·83	—	—	—
20 June	1·53	13·04	22·50	15·98	3·65	8·4	1	—
27 June	2·88	14·62	27·40	11·59	3·89	—	—	—
4 July	4·35	13·22	29·49	11·82	4·24	16·5	4	3
12 July	6·63	12·62	35·85	7·67	3·11	12	3	6·4
25 July	14·15	16·20	41·32	8·04	3·19	—	—	13·0
7 Aug.	21·24	21·42	48·16	6·53	3·04	—	—	13·4
15 Aug.	18·26	30·05	43·32	6·29	2·88	—	—	12·9

* As percentage of fresh weight. † As percentage of dry weight.

TABLE 3. *Radish, Sparkler*; *seed sown* 17 *March* 1944. *Data for the whole plant*

Date	Av. dry weight (g.)	% solids*	% total carbohydrate†	% ash†	% N†	
17 Mar.	0·0074	93·39	21·72	4·41	4·70	Seedlings through Mar. 29
8 Apr.	0·083	7·86	15·31	17·89	5·09	Leaves in rosette, no 'bulb' formed
26 May	2·03	6·24	24·16	14·26	3·85	Marketing stage, flower initial present
30 June	7·47	12·15	24·30	11·26	1·76	Full flowers
26 Sept.	12·46	21·67	19·49	11·40	2·70	Ripe fruit, leaves dropped. Av. of 34·5 fruits and 172·5 seeds per plant

* As percentage of fresh weight. † As percentage of dry weight.

TABLE 4. *Yellow water-lily*, 1943. *Data for the whole plant**

Date	% total carbohydrate†	% ash†	% N†	
30 Jan.	48·71	6·24	2·24	Dormant
15 Apr.	48·40	8·26	2·88	Leaves extending
8 May	48·60	9·80	2·70	Leaves emerged. Flower initials present
26 June	42·27	9·57	2·50	4 floating leaves. 2 flowers
9 Sept.	45·98	10·17	2·85	7 floating leaves. 2 fruits

* Figures are calculated for a rhizome length of 20 cm. with attached roots and one main shoot.
† As percentage of dry weight.

with creeping rootstock. Its distribution of carbohydrate, nitrogen and ash was estimated in an arbitrary portion of the plant consisting of a terminal bud, with 20 cm. length of rhizome, and the roots which grew from it. Most of the roots behind a terminal bud grow from this length of rhizome.

Accepting these considerations, a close correlation was obtained between the time of production of flower initials and a maximum in the curve representing the percentage of total carbohydrate plus the percentage of ash in the whole plant of all six species (Figs. 4 and 5). It made practically no difference to the form of the curves if the percentage of nitrogen was also included. Pea and bean, the large-seeded annuals (Fig. 5), make their flower initials at an early stage in growth of the seedling as mentioned above, and at this stage the plant as a whole, including the old seed, has much greater percentages of carbohydrate plus ash than will occur again in the growth cycle until the production of fruits diverts some of it into certain parts at the expense of others. This therefore amounts to a virtual maximum of carbohydrate plus ash. It is, indeed, a small subsidiary maximum in the pea (Fig. 5). The curve for fumitory in Fig. 4 rises to a maximum and remains approximately at that value, forming flower initials all the time.

An examination of Tables 1–4 and Figs. 2 and 3 will show that there is no correlation between the production of flower initials and the percentages of nitrogen and of ash considered separately. Percentages of total carbohydrate in the whole plant are generally relatively high when flower initials are made, though the correlation is not so close in all species as that between flower initial formation and carbohydrate *plus* ash. This suggests that the amount of carbohydrate is important, however, and would justify earlier work by the writer, where certain flowering reactions were explained by irregularities in carbohydrate supply (Grainger, 1938, 1947).

The observed relation between flower initial formation and the time of maximum amount of carbohydrate plus ash is in line with suggestions made by the writer in a recent review (Grainger, 1943). Changes from vegetative phyllotaxis to floral symmetry were regarded as indications of change in the pressure relations of the growing point. Carbohydrates and soluble salts measured as ash are the two classes of substances which could change the osmotic pressure of the growing point.

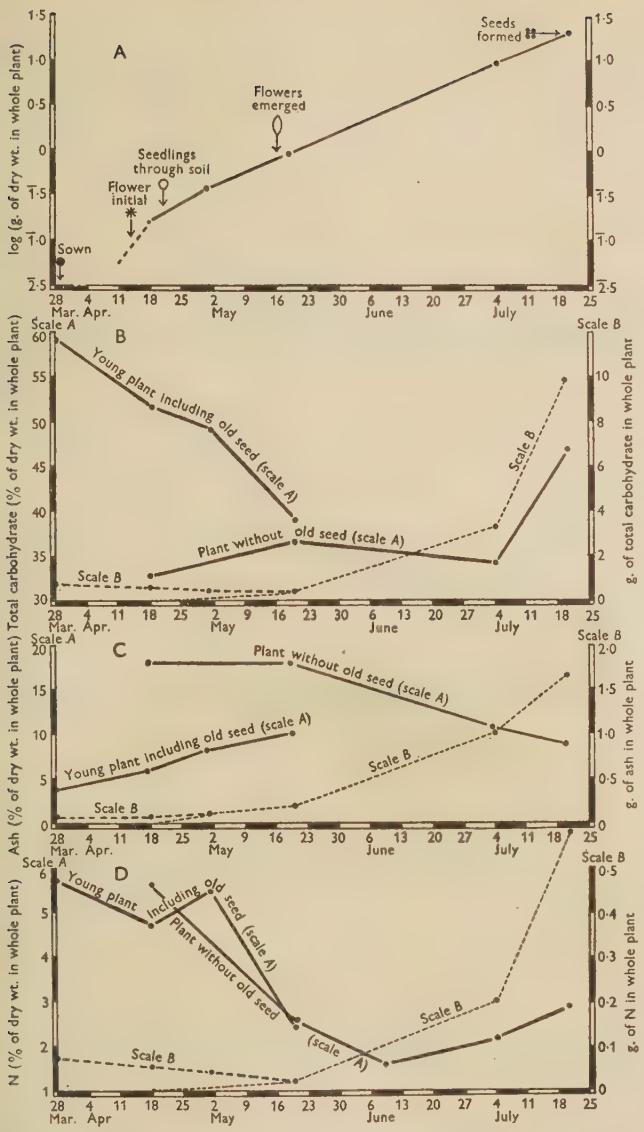
A
Seeds formed
Flowers emerged
Seedlings through soil
Flower initial
Sown
log (g. of dry wt. in whole plant)
Mar. Apr. May June July
B
Young plant including old seed (scale A)
Plant without old seed (scale A)
Scale B
Scale A
Scale B
Total carbohydrate (% of dry wt. in whole plant)
g. of total carbohydrate in whole plant
C
Plant without old seed (scale A)
Young plant including old seed (scale A)
Scale B
Ash (% of dry wt. in whole plant)
g. of ash in whole plant
D
Young plant including old seed (scale A)
Plant without old seed
Scale B
N (% of dry wt. in whole plant)
g. of N in whole plant

Fig. 2. Broad bean. A, increase in dry weight of the whole plant plotted as logarithm of dry weight in grams. B, C and D, percentages and amounts of total carbohydrate, ash and nitrogen respectively in the whole plant. Percentages, reading on scale *A*, are shown by full lines; actual weights, reading on scale *B*, by the broken lines. Percentages and amounts are plotted separately for the young plant including the old seed, and for the plant without the old seed. Actual weights of carbohydrate, ash and nitrogen in the young plant including the old seed are plotted with heavy broken lines; amounts in the plant without the old seed are plotted with light broken lines.

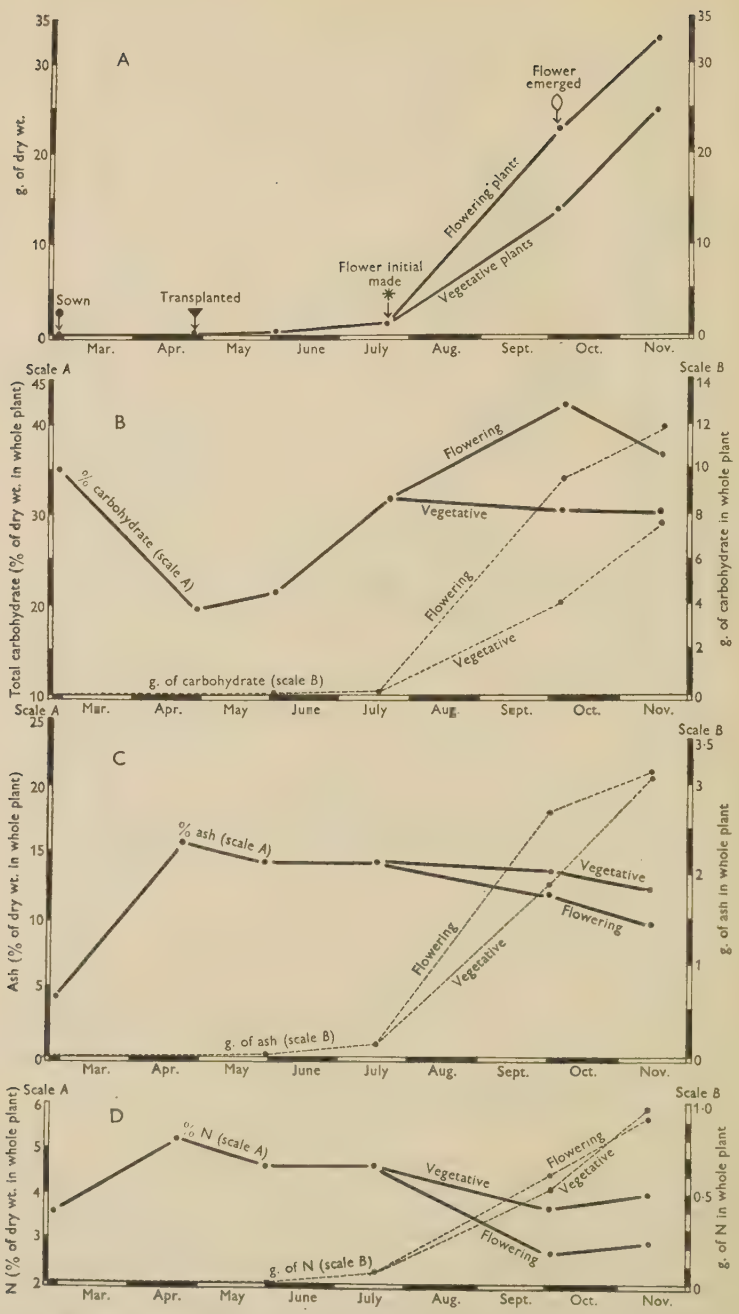

Fig. 3. Leek, variety Musselburgh. A, increase in dry weight of the whole plant. Note the increase in dry weight of flowering plants when compared with vegetative plants between July and November. B, C and D, percentages and amounts of total carbohydrate, ash and nitrogen respectively in the whole plant. Percentages, reading on scale *A*, are shown by full lines; actual weights, reading on scale *B*, by broken lines.

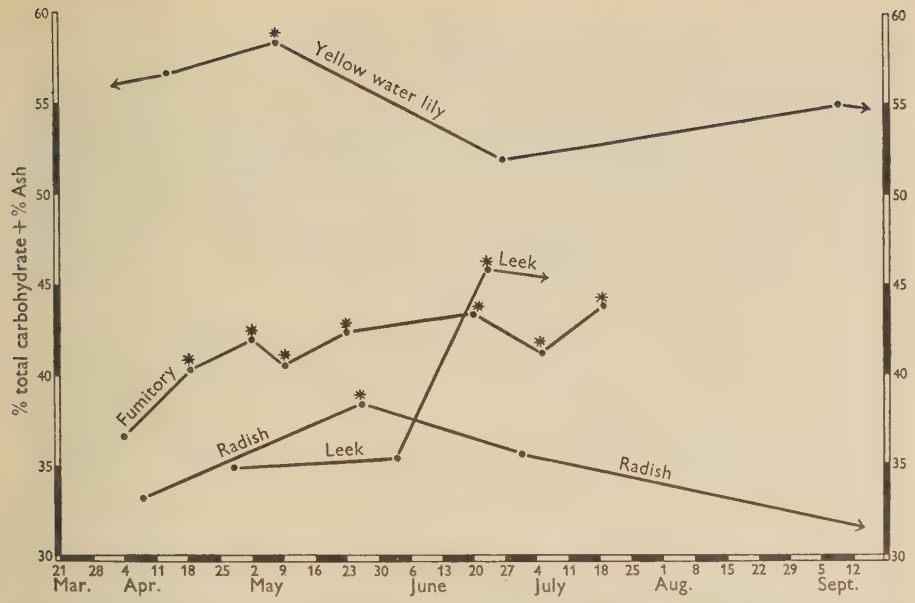
% total carbohydrate + % Ash
Yellow water lily
Leek
Fumitory
Radish
Leek
Radish
21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 5 12
Mar. Apr. May June July Aug. Sept.

Fig. 4. Relation between flower initial formation (shown by asterisks) and percentage of total carbohydrate plus percentage of ash in the whole plant; small-seeded plants and an aquatic perennial.

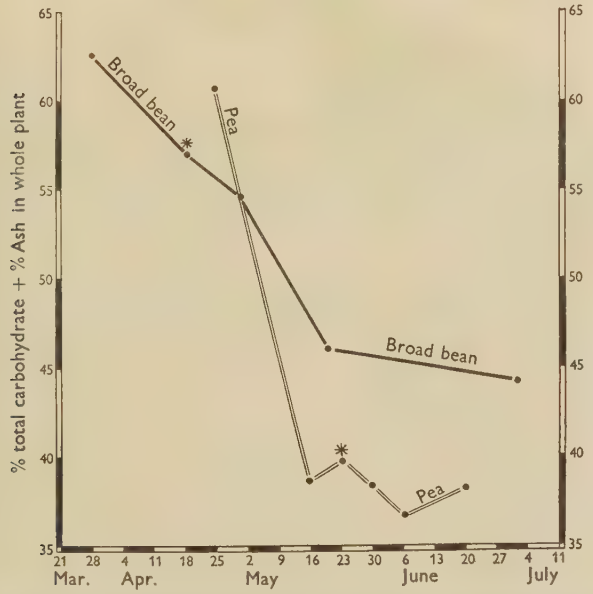
% total carbohydrate + % Ash in whole plant
Broad bean
Pea
Broad bean
Pea
21 28 4 11 18 25 2 9 16 23 30 6 13 20 27 4 11
Mar. Apr. May June July

Fig. 5. Relation between flower initial formation (shown by asterisks) and percentage of total carbohydrate plus the percentage of ash in the whole plant; large-seeded plants.

An interesting contributory point to this idea can be seen in Table 6. Flower initials of the yellow water-lily are sufficiently large for some analyses to be made at several stages of development. The miniature flowers *themselves* have a high content of total carbohydrate, and also relatively high amounts of solid material and nitrogen, at an early stage of formation. It was, unfortunately, not feasible to obtain sufficient material in the earliest stage to estimate the amount of ash, but this would probably be high, corresponding to the high percentage of dry matter.

TABLE 5. *Dry weights in g. of leek. Averages of four plants*

	Roots	Bulb	Leaves	Fl. stalk	Fl. and spathe	Whole plant
10 Dec. 1942 Veg.	0·26	2·37	5·32			7·95
Flg.	0·30	3·67	5·37	4·77	0·48	14·59
13 Feb. 1943 Veg.	0·34	3·53	6·63			10·50
Flg.	0·71	4·50	4·77	4·41	0·43	14·82

TABLE 6. *Changes in flower initials of* Nuphar lutea, *Canon Hall, Huddersfield*

Age of flower initial: months after formation	Dry wt. (g.)	Solids (% of fresh wt.)	Total carbohydrate (% of dry wt.)	Ash (% of dry wt.)	N (% of dry wt.)
2	0·045	10·34	59·00	—	4·85
9	1·10	8·21	30·19	8·11	3·63
12	1·38	7·89	26·81	8·24	2·60

Changes in nutrients after flowering

It has been mentioned that the onset of fruiting causes the accumulation of large quantities of nutrients, and particularly of carbohydrate, in the developing fruits. This is well seen in Fig. 6, which gives the collective results of analyses for percentage of total carbohydrate in the whole plant, for six successive sowings of fumitory. Sowings were made at intervals throughout the year, but the six recorded in Fig. 6 were the only ones which produced significant growth. The percentage of total carbohydrate rises towards the end of the growth period of each sowing (Fig. 6A) and, although it could not be shown visually in the complexity of that figure, the increase, nevertheless, coincided with the appearance of fruits. This fact can be shown (Fig. 6B) by averaging all the curves of Fig. 6A. Non-fruiting plants maintain a percentage of total carbohydrate, round about 25, through the whole period when growth is possible. Inclusion of the fruiting plants produces a significantly higher average percentage of total carbohydrate.

The demand for large amounts of carbohydrate by the developing fruits also coincides with a falling off in the dry-weight curve (Fig. 7). Fumitory makes flower initials with such profusion that there is insufficient carbohydrate for them all. The situation is aggravated in this plant by the fact that the seeds contain 17% of oil or fat. This has a calorific equivalent about $2\frac{1}{2}$ times that of carbohydrate, and has

been so calculated to give the equivalent amounts of carbohydrate in Fig. 6. There can be little doubt that the enormous drain on carbohydrate brings growth of the plant to an end, and so a large number of flower initials never develop, and perish with the plant (Fig. 7).

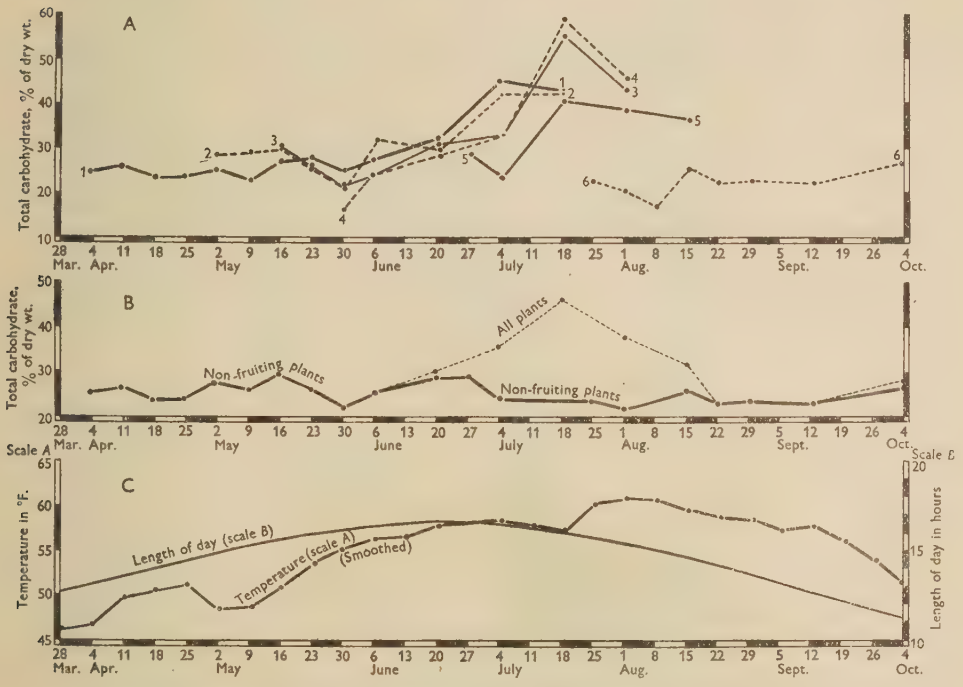
A
Total carbohydrate, % of dry wt.
B
All plants
Non-fruiting plants
Non-fruiting plants
Total carbohydrate, % of dry wt.
Scale A
Scale B
C
Temperature in °F.
Length of day (scale B)
Temperature (scale A) (Smoothed)
Length of day in hours
28 4 11 18 25 2 9 16 23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 5 12 19 26 4
Mar. Apr. May June July Aug. Sept. Oct.

Fig. 6. A, percentages of total carbohydrate in whole plants of fumitory sown successively: (1) sown 1 December; (2) sown 1 February; (3) sown 1 April; (4) sown 1 May; (5) sown 1 June; (6) sown 1 July. No significant growth was obtained from sowings on 1 August, 1 September and 1 November. B, percentage total carbohydrate in the whole plant averaged from the information given in A above. Non-fruiting plants were averaged separately from fruiting plants whenever both were present (from 6 June to 22 August and 12 September to 4 October). C, length of day and temperature during the period covered by A and B.

Leek plants, on the other hand, have significantly greater amounts of dry weight, of carbohydrate, ash, and initially of nitrogen, in the flowering plants than in those which remain vegetative. These differences are striking, but it is not certain, from the present work, whether they represent cause or effect of flowering. It may be that the formation of a flower initial stimulates the plant to greater production of carbohydrate, and greater intake of ash and nitrogen. Equally possible, however, is the view that it is those plants which *earlier* have higher amounts of carbohydrate and ash, which actually make flower initials. Perhaps it is significant that about

one-quarter of the crop under observation 'bolted' into flower, and this percentage is fairly common. It may therefore be that the ability to form a high level of carbohydrate plus ash quickly, and therefore to 'bolt', is a Mendelian recessive character.

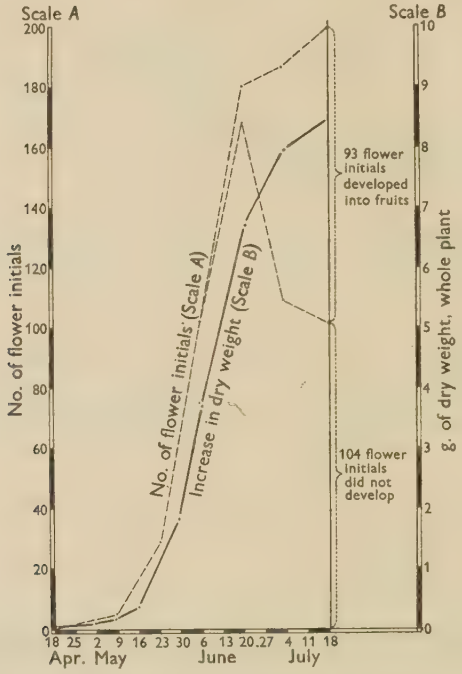
Scale A
Scale B
No. of flower initials
g. of dry weight, whole plant
No. of flower initials (Scale A)
Increase in dry weight (Scale B)
93 flower initials developed into fruits
104 flower initials did not develop
18 25 2 9 16 23 30 6 13 20.27 4 11 18
Apr. May June July

Fig. 7. Average number of flower initials and increase in dry weight, fumitory sown 1 December.

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NITROGEN CONTENT IN RELATION TO FUNGAL GROWTH IN APPLES*

By JOHN COLHOUN
Plant Disease Division, Ministry of Agriculture,
The Queen's University of Belfast

(With 3 Text-figures)

The effects of change in the concentrations of nitrogen, malic acid and sugar in a standard nutrient medium on the rate of spread of *Cytosporina ludibunda* Sacc. and *Fusarium fructigenum* Fr. have been studied.

At low concentrations the rate of radial spread of *Cytosporina ludibunda* falls as nitrogen increases from about zero to 0·03%, whereas at high acid concentrations the rate of spread increases with nitrogen increasing within the same range. At an intermediate concentration of acid spread is unaffected by varying nitrogen content. The response of *Fusarium fructigenum* to nitrogen is similar to that of *Cytosporina ludibunda* except that the acid content at which the reversal of the relationship between radial spread and nitrogen concentration occurs is lower than that for *C. ludibunda*.

The presence of extracts from lentils or apples in media increases the rate of the radial spread of *C. ludibunda* and lowers the acid concentration at which there occurs a reversal from the direct relationship between radial spread and nitrogen content to an inverse relationship.

Introduction

The effects of varying the concentrations of carbohydrate and malic acid at a constant level of nitrogen on the radial spread in media of a number of fungi including *C. ludibunda* Sacc. and *Phomopsis coneglanensis* Trav. have been studied by Seth (1934). Similar experiments were made by Carter (1934) who studied *Fusarium fructigenum* Fr. Colhoun (1940), on repeating a number of experiments made by these workers, reported certain changes in the response of strains of these three fungi to nutrient and suggested that the changes might be due to saltation.

Horne (1938) and Muskett, Horne & Colhoun (1938) have shown that the radial advance of *Cytosporina ludibunda* in apples of the variety Bramley's Seedling is directly correlated with the nitrogen content of the fruit. Horne (1935, 1939) has suggested that when apples of this variety containing relatively low concentrations of acid, or sweet apples of the variety Bascombe's Mystery are inoculated with *C. ludibunda* an inverse relationship between the radial advance of the fungus and the nitrogen content may exist. He has pointed out (1931, 1935) that in analysing the effect of chemical composition on fungal growth it is impossible to obtain exact information by inoculating apples since changes occur in the chemical constituents of the fruit with the passage of time.

* Part of a thesis approved for the Degree of Doctor of Philosophy in the University of London, 1940.

The work of Carter (1934) on the radial spread of *Fusarium fructigenum* in media containing different concentrations of nitrogen provides some information regarding the relationship between nitrogen content and the radial spread of this fungus. She suggested that at low levels of acid the direct relationship between radial spread and nitrogen content may be reversed, but since in her experiments only one carbohydrate content and three concentrations of malic acid were employed it appeared desirable that the problem should receive fuller investigation.

Material and experimental methods

Cytosporina ludibunda strain CE used by Seth (1934) and *Fusarium fructigenum* strain D employed by Carter (1934) were used in this investigation. These strains had been maintained on Brown's synthetic medium (Brown, 1925) in Petri dish cultures from the termination of the investigations made by Seth and Carter until the commencement of the present study in 1937.

The composition of the standard medium used was as follows: glucose (anhydrous) 18·0 g., asparagin 2·0 g., magnesium sulphate 0·75 g., potassium phosphate (tribasic) 1·25 g., agar powder 7·5 g., water 1000 ml. In a few of the early experiments the concentration of agar was 2·0%.

The carbohydrate series of media were prepared as described by Seth (1934), the glucose in the standard medium being replaced by the kind and concentration of sugar required. Acid series were also prepared as described by Seth, malic acid being employed. Series of media of different nitrogen contents were prepared by varying the concentration of asparagin in the standard medium. In one experiment substances such as ammonium nitrate were substituted for asparagin as the source of nitrogen. In certain experiments a crude extract from lentils or apples was added to the media. The extract from lentils was procured by the method described by Buston & Pramanik (1931). The extract from apples was prepared as follows. A quantity of apples was peeled, cored and steamed for 3 hr. with a small quantity of water, the pulp being then squeezed through winceyette. The extract was concentrated to contain about 5% solid matter and saturated at room temperature with barium hydroxide so that it was strongly alkaline to phenolphthalein. The addition of 4 volumes of alcohol gave a heavy precipitate containing organic acids. After standing for 3 days at 0° C. the mixture was filtered and the alcohol removed by distillation under reduced pressure at a temperature below 45° C. The filtrate was neutralized by the passage of carbon dioxide, brought to boiling-point and again neutralized with carbon dioxide. The last traces of barium were removed by the addition of the requisite quantity of sulphuric acid.

In the experimental work Petri dishes were filled with medium to a depth of approximately 0·4 cm. and in preparing sets of plates five replicates were employed. The plates were inoculated and incubated at 20° C. Seth's method of inoculation was employed with *Cytosporina ludibunda* but spore inocula of *Fusarium fructigenum* were used.

The diameter of the colony in each plate was measured at regular intervals of time until the ninth day. Measurements were taken along two diameters at right angles to one another, additional measurements being made if the margin of the colony was irregular. In this paper comparisons of spread are based chiefly on the mean length of radius after 9 days' growth calculated for each set of five plates. Each experiment was made on at least two separate occasions and the results carefully compared.

The hydrogen-ion concentrations of gel media were determined using the glass electrode.

The composition of the medium is indicated in the text by a figure representing the percentage concentration of the varying constituent. Thus 4·0 G represents 4·0% glucose, 6·5 F represents 6·5% fructose, 2·0 S represents 2·0% sucrose, 0·6 MA represents 0·6% malic acid and 0·04 N represents 0·04% nitrogen calculated from the weight of asparagin in the medium.

RADIAL SPREAD IN DIFFERENT CONCENTRATIONS OF NITROGEN

(*a*) Cytosporina ludibunda

Series of media were prepared employing 13·5 and 4·0 G in conjunction with 0·6 MA and eight concentrations of asparagin (0·0, 0·005, 0·01, 0·02, 0·03, 0·04, 0·05 and 0·06 N). Series were also prepared using two mixtures of sugar, 2·0 G + 5·0 F + 1·0 S and 2·0 G + 7·0 F + 3·0 S, with the same concentrations of malic acid and asparagin as were used in the experiments with glucose. In these experiments the media contained 2·0% agar. The results obtained did not suggest the existence of a relationship between radial spread and nitrogen content. Similar results were obtained when the same concentrations of asparagin were employed with 13·5 or 4·0 G in conjunction with 0·3 or 0·8 MA.

When ammonium nitrate, potassium nitrate or calcium nitrate was substituted for asparagin as the source of nitrogen it was found that at 13·5 G and 0·6 MA the radial spread of the fungus in 9 days was reduced in all cases when the nitrogen content was increased from 0·02 to 0·06 N.

Analysis of the agar powder used showed it to contain 0·25% nitrogen. Since it was considered that failure to demonstrate a marked effect on radial spread by varying the asparagin content might be due to the nitrogen in the agar an experiment was made employing a nutrient solution, as in the standard medium but without agar. In this test 13·5 G and 0·6 MA were employed in conjunction with the eight concentrations of asparagin previously employed. The dishes were examined 9 days after inoculation, but since the colonies broke up when the dishes were moved it was not possible to make accurate measurements of spread. Growth was very poor at 0·0 N, much better at 0·005 N, and good at all higher concentrations of nitrogen. In view of these results it was decided to reduce the quantity of agar in the media used in subsequent experiments from 2·0 to 0·75%.

Series of media were prepared using 13·5 G in conjunction with every combination of 0·1, 0·2, 0·4, 0·5, 0·6, 0·8 and 1·0 MA and seven concentrations of asparagin (0·0, 0·01, 0·02, 0·03, 0·04, 0·05 and 0·06 N). The mean values of radial spread (cm. in 9 days) obtained for each set of replicates are expressed graphically in Fig. 1, where radial spread is plotted against nitrogen concentration for each acid content.

It should be noted from Fig. 1 that at 0·1, 0·2 and 0·4 MA, with nitrogen increasing between 0·0 and 0·03 N, the rate of spread falls, but with nitrogen increasing between 0·03 and 0·06 N the rate remains relatively constant. At 0·5 MA radial

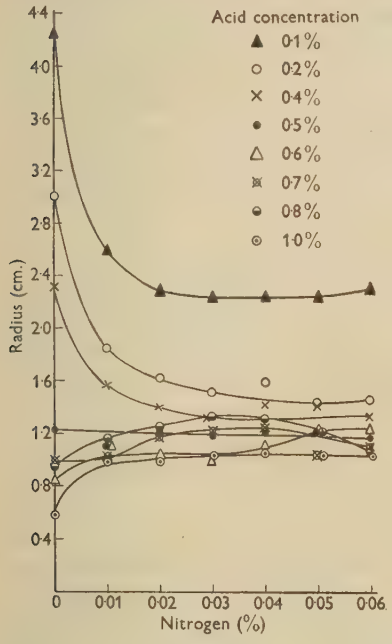

Fig. 1. Radial spread of *Cytosporina ludibunda* in relation to nitrogen content for eight concentrations of malic acid using 13·5 G.

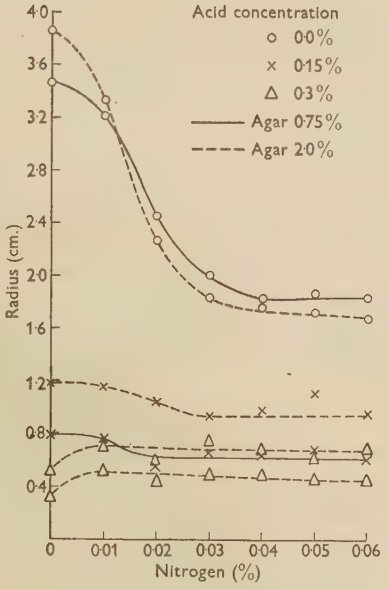

Fig. 2. Radial spread of *Fusarium fructigenum* in relation to nitrogen content for three concentrations of malic acid using 12·0 G.

spread is very little affected by varying nitrogen content between 0·0 and 0·06 N. At 0·6, 0·7, 0·8 and 1·0 MA the rate of spread increases with nitrogen increasing from 0·0 to 0·03 N, but further additions of nitrogen up to 0·06 N have little effect on the rate.

One experiment was made to ascertain if the effect of nitrogen content on radial spread was materially affected by the concentration of glucose. Series of media were prepared employing every combination of the seven concentrations of asparagin used in the previous experiment and 0·4, 0·6 and 0·8 MA with 6·0 and 9·0 G. When the mean values of radial spread (cm. in 9 days) obtained were plotted against

nitrogen content for each acid concentration it was found that the shapes of the curves were similar to those for the corresponding acid contents at 13·5 G.

The effect of nitrogen content on the radial spread of the fungus in media containing glucose, fructose and sucrose in the ratio 2·0 G, 6·5 F and 2·0 S was also studied employing the same concentrations of malic acid and asparagin as in the previous experiment. The results obtained are in agreement with those presented for radial spread in media where glucose alone was the source of carbohydrate.

(*b*) Fusarium fructigenum

The seven concentrations of asparagin used in experiments with *Cytosporina ludibunda* were again employed in combination with 12·0 G and three acid contents (0·0, 0·15 and 0·3 MA). The experiment was repeated using 2·0% agar in the media in order that the results obtained might be compared with those stated by Carter (1934) who carried out a similar experiment. The mean values obtained in each case for radial spread (cm. in 9 days) are given in Fig. 2 where radial spread is plotted against nitrogen concentration for each acid content.

From Fig. 2 it can be seen that the shape of the curve at any acid concentration is not affected by the amount of agar present in the media. At 0·0 and 0·15 MA the rate of spread falls with nitrogen increasing from 0·0 to 0·03 N, but with further increases there is little change in the rate. At 0·3 MA an increase in the rate of spread occurs with nitrogen increasing from 0·0 to 0·01 N but with further additions of nitrogen above 0·01 N the rate is unaffected.

Radial spread in different concentrations of nitrogen in the presence of extracts from lentils or apples

The experiments already described have shown that the radial spread of *C. ludibunda* in media is related to nitrogen content. When the results obtained are compared with those derived from experiments where apples were inoculated with this fungus (Horne, 1938; Muskett *et al.* 1938) it is obvious that varying the nitrogen content does not affect the spread of the fungus in media to as great an extent as it influences the radial advance in apples. Since synthetic media were used in these experiments it was decided to investigate the response of *C. ludibunda* to nitrogen content in media containing a small quantity of an extract from lentils or apples. The extract from lentils was used because it had been shown by Buston & Pramanik (1931) to contain a growth-promoting substance.

Series of media were prepared employing 0·1, 0·4, 0·6 and 0·8 MA with 13·5 G and the seven concentrations of asparagin previously used. Where apple or lentil extract was employed sufficient was added to the media to provide 0·1% (dry weight) of the extract. The results obtained for the mean values of radial spread (cm. in 5 days) for each set of replicates in series with apple extract, lentil extract or no extract are presented graphically in Fig. 3 where radial spread is plotted against nitrogen content for each acid concentration.

From Fig. 3 it is seen that the effect of nitrogen content on radial spread is marked only at low concentrations of nitrogen. In the absence of lentil or apple extract, with nitrogen increasing from 0·0 to 0·02 N there is a fall in the rate of spread at 0·1 MA, while at 0·4 MA the rate is unaffected and at 0·6 or 0·8 MA there is an increase in the rate. With nitrogen increasing within the above range and with lentil extract present the rate shows an increase at all acid contents. In the presence of apple extract an increase in the rate occurs at 0·4, 0·6 and 0·8 MA with nitrogen increasing between 0·0 and 0·02 N, but at 0·1 MA the rate is unaffected by varying nitrogen content. When increasing nitrogen content brings about an increase in the rate of spread this increase is usually greater in the presence of either extract than

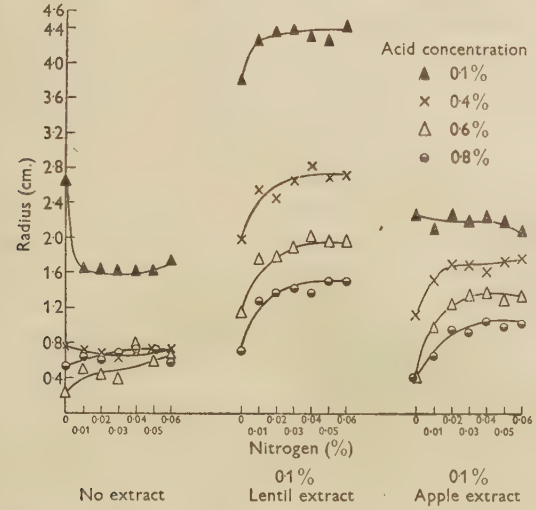

Fig. 3. Radial spread of *Cytosporina ludibunda* in relation to nitrogen content for four concentrations of malic acid in media with apple or lentil extract or with no extract.

in its absence. The rates of spread are also usually greater in media containing lentil or apple extract than those in the corresponding media without extract.

It was possible to make measurements of radial spread in all media except that at 0·1 MA with lentil extract on both the seventh and ninth days after inoculation. The only difference observed between the results obtained for the values on the fifth day and on the later occasions was that at 0·4 MA without extract there was a fall in the rate of spread on both the seventh and ninth days with nitrogen content increasing between 0·0 and 0·02 N whereas on the fifth day the rate was unaffected.

One experiment was made employing sugars in the ratio 2·0 G, 6·5 F, 2·0 S combined with 0·4 and 0·6 MA and the seven concentrations of asparagin previously used. One series of media contained no extract while two similar series contained 0·1% (dry weight) of lentil and apple extracts respectively. The mean

values of radial spread (cm. in 9 days) obtained show that the response of the fungus to nitrogen in media containing the combined sugars is similar to that when glucose alone is used.

The effects of varying the concentrations of extracts from lentils and apples were studied in one experiment. Series of media were prepared employing 13·5 G with 0·1 MA and three concentrations of asparagin providing 0·0, 0·01 and 0·02 N. The concentrations of the extracts used provided 0·0, 0·025, 0·05 and 0·1 % (dry weight). The mean values of radial spread (cm. in 5 days) obtained for each set of replicates show that the addition of lentil or apple extract to the media is accompanied by an increase in the rate of spread, the increase in the rate being usually dependent on the concentration of the extract. The addition of lentil extract caused a greater increase in the rate of spread than an equal concentration of apple extract. With nitrogen increasing from 0·0 to 0·01 N there is a fall in the rate in the absence of extract or in the presence of 0·05 or 0·025 % apple extract or 0·025 % lentil extract but an increase in the rate occurs in the presence of 0·1 % lentil extract. With 0·1 % apple extract or 0·05 % lentil extract present the rate is relatively unaffected by varying the nitrogen content.

The addition of extracts from lentils or apples involves adding a small amount of sugar to the media, but the results obtained during the investigation did not suggest that increases in the sugar content of the media are generally accompanied by large increases in the rate of spread of *C. ludibunda*. An experiment was, however, made to compare the effect on the rate of spread of small increases in the sugar content of the media with that brought about by the addition of either extract. Employing 0·1 MA and 13·5 G it was found that in the presence of 0·1 % apple extract the radial spread of *C. ludibunda* in 5 days was double that in media without extract. The addition of a similar concentration of lentil extract trebled the radial spread, whereas it was unaffected by the addition of any of the following concentrations of sugar: 0·1 G, or 0·1 F or 0·18 S or 0·02 G, 0·06 F and 0·02 S.

The amount of titratable acid present in 100 ml. of 0·1 % lentil or apple extract was found to be negligible. Employing 13·5 G with 0·0, 0·01 and 0·02 N it was shown that the addition of 0·1 % lentil or apple extract had very little effect on the pH of the media at 0·1 MA and no effect at 0·4 MA. The results obtained demonstrate that the effect of the addition of one of the extracts on the concentration of malic acid at which the change from the direct to the inverse relationship between radial spread and nitrogen content occurs cannot be attributed to the extract rendering the medium more acid.

Discussion

In this investigation the effect of increasing the concentration of nitrogen, supplied in the form of asparagin, above the amount (about 0·002 %) present in the standard medium without asparagin was studied. With nitrogen increasing between 0·002 and 0·03 % and with acid and sugar contents remaining constant the rate of spread of *C. ludibunda* falls at low acid concentrations, remains fairly constant at medium

acid concentrations whilst it increases at high concentrations of acid. With nitrogen content increasing between 0·03 and 0·06% the rate of spread remains relatively unaltered at all acid concentrations. The response of the fungus to nitrogen appears to be only slightly affected by variations in the concentration or kind of sugar present.

The results obtained with *Fusarium fructigenum* differ from those recorded by Carter (1934) in that the increase in the rate of spread associated with increasing nitrogen which she found at low levels of nitrogen in the absence of acid or with 0·15% acid was not observed. The response of *F. fructigenum* is now found to be similar to that of *Cytosporina ludibunda*, but the level of acid at which the reversal in the relationship between nitrogen content and radial spread occurs is lower than that with *C. ludibunda*.

It has been found that the presence of extracts from lentils or apples in media has a marked differential effect on the response of *C. ludibunda* to nitrogen content. The effect of these extracts, apart from increasing the rate of spread, is to lower the acid concentration at which a change from the direct relationship between spread and nitrogen content occurs. This alteration in the acid concentration at which this reversal or change takes place cannot be explained on the grounds of the pH of the media being altered by the addition of the extract.

The nitrogen content of apples is very variable, depending on such factors as the manurial treatment given to the trees or the number of apples borne by individual trees. Horne (1938), working with fruit from trees on East Malling rootstocks receiving varied manurial treatments, found that a positive and highly significant correlation existed between the radial advance of *C. ludibunda* and the nitrogen content of the fruit. The apples used by Horne were of the variety Bramley's Seedling and showed mean acid content ranging from 0·546 to 0·756% at the time when the inoculations were made. Similar results were obtained by Muskett *et al.* (1938), who have given records of the nitrogen content of apples from groups of similar trees receiving varied manurial treatment. These workers found that in one year the nitrogen content of the fruit ranged from 0·0218, when the trees received no treatment, to 0·0619% when the trees received sulphate of ammonia and muriate of potash. Muskett *et al.* (1938) also found that the nitrogen content of the fruit was directly correlated with the radial advance of *C. ludibunda*, but did not record the acid content of the apples which may be presumed to be high since the inoculations were made in December and the variety of apples was Bramley's Seedling.

Horne (1935) has obtained further results which suggest that at relatively low levels of acid an inverse relationship between the radial advance of *C. ludibunda* in apples and nitrogen content may exist. Thus with Bramley's Seedling apples showing average acid content ranging from 0·526 to 0·678% the partial correlations for radial advance and nitrogen content eliminating the effects of acid and fructose were negative and one correlation was highly significant. With average acid content ranging from 0·693 to 1·017% positive partial correlations were obtained, one correlation being significant.

Evidence of a reversal in the response of fungal spread to nitrogen content associated with variety of apple and correspondingly with acid content is forthcoming from results obtained by Horne (1939) during the course of inoculation experiments with apples from trees injected at East Malling with nitrogenous substances according to the methods described by Roach (1934). The injection treatment induced susceptibility of the fruit to attack by *C. ludibunda* in the case of Bramley's Seedling apples and resistance in the case of sweeter apples of the variety Bascombe's Mystery.

The response of *C. ludibunda* to nitrogen content when inoculations are made in apples shows considerable agreement with that in media. Thus it has been found that in apples and in media with low nitrogen contents, a direct relationship between nitrogen content and the radial advance or spread of the fungus exists at high concentrations of acid, whereas at low levels of acid this relationship is reversed. The agreement is not, however, complete, since it has been found that with the nitrogen content of media increasing between 0·03 and 0·06% the spread of the fungus was relatively unaffected at all concentrations of acid. Results obtained from numerous experiments made with apples (Horne, 1931, 1936, 1938; Muskett *et al.* 1938) show that radial advance is greatly affected when the nitrogen content of the fruit is increased above 0·03%. This difference may be due to the fungus being able to utilize only a portion of the nitrogen present in the fruit, or to asparagin being used as the sole source of nitrogen in the media. In this connexion it must be pointed out that Hulme & Roach (1936) found that increases in nitrogen content brought about in apples by injecting nitrogenous compounds into comparable trees were due mainly to increases in the asparagin nitrogen present.

The rate of spread of *C. ludibunda* in media does not show as large increases as have been recorded for radial advance in apples when the nitrogen content increases within the same range. When lentil or apple extract is present in the media a greater increase in the rate of spread occurs when the nitrogen content is increased between 0·002 and 0·03% than is found to occur in the absence of either extract. The addition of such extracts does not, however, cause increases in the rate of spread in media with nitrogen increasing above 0·03%.

The writer wishes to express his thanks to Dr A. S. Horne for his help and criticism, to Prof. V. H. Blackman and Prof. W. Brown for their interest and for the provision of facilities at the Imperial College of Science, London, where the work was carried out, and to Prof. A. E. Muskett for his constant interest and encouragement. The investigation was undertaken during the tenure of a Senior Scientific Research Grant from the Ministry of Education, Northern Ireland.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

A Conversazione on recent advances in applied entomology, mycology and plant pathology was held on Friday, 23 July 1948, in the Department of Botany (by kind permission of Prof. W. Brown), Imperial College of Science and Technology, London.

About 400 people attended, including some 70 delegates to the Commonwealth entomological and mycological conferences.

List of Exhibits

A.R.C. Plant Virus Research Unit, Molteno Institute, Cambridge

Recent work on viruses and virus diseases. — Roy Markham, R. E. F. Matthews, J. D. Smith, Kenneth M. Smith and G. R. Wyatt

A.R.C. Unit of Insect Physiology, Cambridge

Research in insect egg-shells. — J. W. L. Beament
The action of inert mineral dusts on insects. — V. B. Wigglesworth
Insecticidal action. — W. A. David
Aphid behaviour. — J. S. Kennedy

A.R.C. at Sutton Bonington

Potato dry rot. — A. R. Wilson and R. K. McKee

British Museum (Natural History)

Biological material preserved in methyl methacrylate ('Perspex'). — J. P. Doncaster

East Malling Research Station

Mineral deficiencies: diagnosis and cure. — W. A. Roach
Strawberry and raspberry viruses. — I. W. Prentice and C. H. Cadman
Verticillium spp. — W. G. Keyworth
Raspberry cane midge and blight, a combined insect and fungus attack. — R. S. Pitcher and P. C. R. Webb
Fruit tree red spider mite. — A. M. Massee and M. D. Austin

Experimental and Research Station, Cheshunt

Diseases of glasshouse plants. — W. F. Bewley and P. H. Williams
Greenhouse whitefly and its parasite *Encarsia*. — E. R. Speyer

Forest Products Research Laboratory, Princes Risborough

Damage by the house longhorn beetle, *Hylotrupes bajulus*, in Great Britain. — E. C. Harris and Joyce E. Green
Defects caused by insects and fungi in colonial timbers and their identification. — E. C. Harris, Joyce E. Green, W. P. K. Findlay and R. C. Fisher

Imperial College of Science and Technology, South Kensington

The effect of pre-soaking on the germination of seed of *Phaseolus vulgaris*. JOCELYN WINFIELD

Leafy galls (*Corynebacterium fascians*). S. E. JACOBS and MARGARET S. LACEY

Indian Forestry Institute, Dehra Dun

Indian forest fungi. K. BAGCHEE

Long Ashton Research Station, nr. Bristol

Systemic insecticides. S. H. BENNETT and H. MARTIN

Spray nozzles, their assembly and uses. H. G. H. KEARNS and N. G. MORGAN

Ministry of Agriculture & Fisheries, Infestation Division, London

The control of rats and mice and of insect and mite pests of food. J. A. FREEMAN

Ministry of Agriculture & Fisheries, Plant Pathology Laboratory, Harpenden

Colorado beetle. I. THOMAS

Seed-borne diseases in relation to seed inspection schemes. F. JOAN MOORE

Mushroom Research Association, Yaxley

Some mushroom diseases. C. J. LA TOUCHE and F. C. ATKINS

Growth rate of mushroom sporophores. R. L. EDWARDS

Slide traps for soil fungi. C. J. LA TOUCHE

Effect of fertilizers on temperature and loss of nitrogen in straw composts. R. L. EDWARDS and S. BURROWS

Selective action of culture filtrates on organisms causing diseases of cultivated mushrooms. C. J. LA TOUCHE, S. BURROWS and R. L. EDWARDS

A design for experiments on mushroom composts. R. L. EDWARDS

Compost colloids. S. BURROWS

N.A.A.S., Bristol

Diseases of field beans. MRS M. JUSTHAM (M. MUNRO) and L. OGILVIE

Black rust of wheat. MRS F. W. M. COLLETT and L. OGILVIE

Home-made dusting machines for farmers and growers. L. N. STANILAND and J. MAYOR

N.A.A.S., Cambridge

Glass models of fungi.

Barley disorder 1947.

Bud proliferation of potatoes.

Growth curves of potatoes, etc.

W. A. R. DILLON WESTON, E. C. LARGE and R. E. TAYLOR

North of Scotland College of Agriculture, Aberdeen

Sheep ticks. G. D. MORISON

Some interesting diseases in North Scotland. ELIZABETH G. GRAY and A. KERR

Pest Infestation Laboratory, Slough

Pre-treatment of building materials to increase persistence of insecticidal films. P. S. HEWLETT and E. A. PARKIN

Plant Pathology Service, Department of Agriculture for Scotland

Pathology of seed production. MARY NOBLE

Queen's University, Belfast

Seed-borne parasitic fungi; seed disinfection.
A. E. MUSKETT, J. COLHOUN, ABDUL ARIF and ABDUS SALAM

Rothamsted Experimental Station, Harpenden

Traps for air-borne spores. P. H. GREGORY
Aphid flight at different relative humidities. L. BROADBENT
The use of barrage balloons and suction traps in sampling aerial populations of insects.
C. G. JOHNSON
Structure of insect cuticle.
Method for evaluation of stomach poisons.
Method for study of effect of insecticides on insect respiration.
M. J. WAY and K. A. LORD
Quantitative estimation of soil micro-organisms influencing soil structure.
P. C. T. JONES, JANET E. MOLLISON and R. J. SWABY
Plant parasitic eelworms. B. G. PETERS, D. W. FENWICK and J. B. GOODEY

Royal Horticultural Society Laboratories, Wisley

The relation of the bean-spindle aphid *Aphis fabae* Scop. to cultivated species of *Euonymus*.
G. FOX WILSON

The University, Birmingham

Red core disease of strawberries. C. J. HICKMAN and MARY P. ENGLISH

The University, Durham

Current work on sheep blowflies. J. B. CRAGG and L. DAVIES

The University, Edinburgh

Scottish Culicoides (blood-sucking midges) and Tabanidae.
A. E. CAMERON, MR MCHARDY and MR CAMPBELL

A.R.C.—Agricultural Research Council.
N.A.A.S.—National Agricultural Advisory Service.

REVIEWS

Animal Colour Changes and their Neurohumours. A Survey of Investigations (1910–43). By G. H. PARKER. Pp. x + 377. Cambridge University Press. 1948. 30*s.*

In 1914 Fuchs's comprehensive account of colour changes produced through the agency of chromatophores appeared in Winterstein's *Handbuch der Vergleichenden Physiologie.* Now, after an interval of thirty years, G. H. Parker has attempted to summarize the additions which have been made to our knowledge in this field of research. No one could be better qualified for the task. Prof. Parker's initial publications on colour change were referred to by Fuchs and, since that date, he and his colleagues at Harvard have made many contributions to the subjects.

In an introductory chapter various types of chromatophores are defined and methods of study described. A chapter of some twenty pages is devoted to colour changes in cephalopods and the section on crustacean chromatophores is only slightly longer. The remainder of the book is concerned with colour changes in vertebrates.

The course of investigation since Fuchs's survey has been largely conditioned by two advances in physiological knowledge; the discovery of hormones, and the chemical transmission of the nervous impulse. In 1914 it was generally considered that chromatophores were under nervous control. Work since that date has shown that in many cases nerve connexions are totally unimportant, colour changes being produced by the action of hormones. Further, where nerves do play a part, the passage of nervous impulse to colour unit is produced by a chemical transmitter, or, as Parker prefers to call such a substance, a neurohumour.

As the author points out, the book is a review, and a considerable amount of data have been moulded into a very orderly arranged and readable text. The attention given to the subject is indicated by the literature list of 1200 titles. This bibliography alone, makes the work an invaluable asset to the zoologist looking for information on chromatophore structure and function.

J. B. CRAGG

Plant Viruses. By KENNETH M. SMITH. Pp. 78. Methuen and Co., Ltd. 2nd ed. 1948. 6*s.*

Although claimed merely as a second edition of Dr Kenneth M. Smith's earlier book, *Plant Viruses* is in fact an entirely new work. No doubt as a result of the progress made in the study of viruses during the thirteen years that have elapsed since the publication of the first edition, the emphasis has been shifted from techniques and experimental procedure to the nature, properties and behaviour of the viruses themselves. This small book provides a balanced and up-to-date introduction to plant viruses for students with some knowledge of biology. The informal style is easy to read, but sometimes leads to ambiguities. Oversimplification and condensation may cause the reader to puzzle over some apparent *non sequitur*, such as the connexion between chlorophyll and respiration on p. 14. The illustrations are excellent.

P. H. GREGORY

Gall Midges of Economic Importance. Vol. III: Fruit. By H. F. BARNES. Pp. 184. Agricultural and Horticultural Handbooks. London: Crosby, Lockwood and Son, Ltd. 1946. 15*s*.

In Vol. III of this series Dr Barnes has maintained the high standard set in Vols. I and II. Emphasis is again laid on biology and control, and where midges are found to differ in their behaviour *in the field* Dr Barnes has treated them as distinct species because 'from the practical point of view, a pest is distinct if it will not attack two different crops growing together even if under experimental conditions it can be made to breed on both'.

Those familiar with Dr Barnes's methods have been led to expect the most meticulous and accurate marshalling of data and this work is no exception; out of 467 references cited only 24 were not seen by the author in the original and, as in Vols. I and II, there is once again the same full acknowledgement given to all workers on midges throughout the world.

The fruit gall midges of economic importance in Great Britain are very fully dealt with and the information is recent enough to include reference to D.D.T. insecticides where they have been found to be effective. For some of the more important midges the references to control measures are indeed so full that it is occasionally difficult to sort out and arrive at the most practical and economic measures.

The few minor errors noted detract little from the value of the work: on p. 59 'Ledbury, Kent' should almost certainly read 'Ledbury, Hereford'; on pp. 81 and 82 the Common Elder is given as the common name for both *Sambucus canadensis* and *S. nigra*, and *S. pubens* is called the Red-Berried Elder which is generally the name given to *S. racemosa*; on p. 120 'Pitmaster' is given for 'Pitmaston' and 'Vicar of Wakefield' for 'Vicar of Winkfield'—a common error.

Having seen three Volumes of this Monograph it is possible to say that it will become the standard reference work on Gall Midges for very many years. All economic entomologists, fruit specialists and progressive growers will constantly need to refer to Vol. III which is very fully indexed and well illustrated. Vol. IV, *Gall Midges of Ornamental Plants and Shrubs*, is shortly to appear and Vols. V-VIII are in preparation. I. THOMAS